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HAMILTON B. G. ROBINSON

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THE EDITOR'S VIEWPOINT

The JOURNAL has a new publisher beginning with this issue. The JOURNAL is still owned and copyrighted by the International Association for Dental Research. As you will notice, the format is essentially the same. Your Editor decided that this is an appropriate time to carry out a few changes that he had been considering for some time. The cover has undergone a face lifting. The paper is now all glossy. A few internal changes such as using superior numbers for references have been instituted. We believe that you will find the JOURNAL essentially the same as that you have read since 1919.

Krogh (p. 3) compared the incidence of dry socket in 773 mandibular molars and premolar extractions treated with sulfonamide tablets with the incidence of dry socket in 2,126 morphologically similar tooth extractions. He found no typical dry sockets in the 66 extractions of unerupted teeth and 707 extractions of erupted teeth when sulfonamide therapy was used. By comparison, he had dry sockets in 27.6 per cent of 225 extractions of unerupted teeth and 3.1 per cent dry sockets in extractions of 1,901 erupted teeth, without sulfonamide therapy.

Stewart (p. 24) measured the root canal volume of the teeth and determined the quantity of fluid, i.e., medication, that might be used in each tooth. To the endodontist using antibiotics measured in units per volume, the tables presented should be clinically important.

Martin (p. 27) gives the first report of the Evanston Dental Caries Study, jointly supported by the Evanston Health Department, the Zoller Memorial Clinic of the University of Chicago, and the Illinois State Health Department. The complete manner in which this study is being conducted should serve as an example to those who may wish to plunge into a fluorinated water program as a so-called demonstration project. Food, teeth, and dental cements, bones from fetuses, and the water supply at various points have been and are being analyzed. This is only one phase of the investigation which is under way. The results found by Martin are most interesting. Even if the caries-fluorinated water concept should prove of little value, the experimental results should advance knowledge sufficiently to justify the project.

McClure (p. 34) summarized the results in terms of dental caries of rats of various substances added to drinking water. Included were sodium sulfate, magnesium chloride, calcium chloride, iodide, thioglycolic acid, silver nitrate, potassium thiocyanate, iron, copper, zinc, urea, penicillin, and nitrate. The study indicates that of this group only urea, nitrate, penicillin, and iodoacetate gave indications of caries inhibition, while there was some suggestion that thioglycolic acid may accelerate the process. Some may criticize this type of investigation as empirical or "shotgun" research. We cannot go along with such criticism. The many substances were selected on the basis of real or imagined relationships suggested by previous investigators. One statement that we do not believe to be true is "current acid decalcification theories suggest that the severity of caries would be reduced by the presence of so much alkali in the rat's oral cavity." The current acid decalcification theory proposes that the important acid production is that in small or microscopic areas beneath plaques and that the pH of the oral cavity probably has little or no effect on these areas, depending on the penetrability of the individual plaque and the penetrating power of substances in the mouth.

Wakeman, Smith, Sarles, Phillips, and Elvehjem (p. 41) give a new method for quantitative determination of microorganisms on rat teeth. Unfortunately, the method requires removal of three teeth before determination of the flora, precluding the possibility of observing changes in the flora from time to time in a single animal. In view of the lack of any other good quantitative method it should prove of value to investigators of rat caries. Comparisons of counts made from extracted human teeth by this method with salivary lactobacillus counts might yield useful information on the problem of the accuracy of salivary counts.

Van Huysen and Muhler (p. 46) report on the effect of stannous fluoride on enamel solubility. One point in their premise, that the upper anterior teeth are protected more by fluoride in drinking water passing over them, has been suggested repeatedly without, we believe, a careful consideration of facts. A thorough study of Arnim's compilation of Leigh and Hyatt's data (*J. D. Res.* 21: 243, 1942) indicates that teeth have different "susceptibilities" within the same mouth and it appears to us that the apparent reduction in caries of these teeth is merely because the level of reduction of susceptibility of the individual is usually at about this point, i.e., where the maxillary anterior teeth are protected. We would like to see clinical studies of the theory of Van Huysen and Muhler as solubility studies on powdered enamel may not be comparable to effects on intact enamel surfaces and fluorides may act as enzyme poisons rather than, or as well as, inhibitors of enamel solution.

Rae and Clegg present two chemical studies supported by grants for the National Research Council of Canada. The first (p. 52) on changes in Ca and P concentrations of saliva shaken with tricalcium phosphate suggests that pH changes in the saliva or solutions is important in determining Ca and P changes. Since the caries susceptibility test of Fosdick, Hansen, and Epple utilizes the effect of incubated saliva on powdered enamel (i.e., Ca-P compound) this result may add to the jigsaw puzzle on caries. The second study indicates that any salt whose anions form an insoluble calcium salt or whose cations form an insoluble phosphate salt will reduce solubility of tooth enamel. This study, and others, seem to accept reduction in enamel solubility as the chief effect of fluorides in caries reduction. We believe that the recent studies of Galagan and Knudson (*Pub. Health Rep.* 62: 1477, 1947) and of Bibby and DeRoche (*J. D. Res.* 26: 446, 1947) suggest that enamel solubility is far from the whole story. Clinically, their results were not comparable to those to be expected from use of a lead fluoride. Perhaps, the enzyme poison effect of fluorides are more important than their effects on tooth solubility and it was this property that led Miller (*Proc. Soc. Exper. Biol. & Med.* 39: 389, 1938) to use fluorides to inhibit caries.

Cheyne and Horne (p. 58), from a detailed study of 429 children, present additional evidence that a complete dental examination includes radiographic studies. They show that neither radiographic nor clinical examinations are complete in themselves but that they augment each other. Without bite-wing radiographs, approximately one-third of the cavities were missed. It should be evident to dentists that x-ray examination is a part of any adequate routine dental study.

Glickman (p. 9) has studied periodontal tissue changes in vitamin C-deficient guinea pigs and finds that in spite of edema, hemorrhage, and degeneration of the gingivae, gingivitis is not increased over control animals. Another term for the periodontal changes, he finds, is periodontosis. As in periodontosis, pocket formation is not a usual result of vitamin C deficiency.

The Proceedings of the New York Section of the International Association for Dental Research appear on page 68. We welcome well-organized, brief records of the proceedings of our various sections.

During September, 1947, the University of Michigan sponsored and played host to a dental caries conference. The "faculty" included imported caries investigators from schools and health service institutions from New England to California. Not only were formal papers presented, but a spirited discussion was encouraged and recorded. After five days of work, the participants reported as committees on different phases of the problem. We felt that this "inservice training course" was one of the outstanding contributions to dental research. As the material had been collected and organized, we believed that it should be well disseminated. Two telephone calls set the ball in motion, one to Ann Arbor to find if the University of Michigan would agree and the other to ask cooperation of our publisher. As a result, the Proceedings will be published in our February, April, and June issues with 150 extra pages allocated by the publisher. Personally, we hope the Proceedings of the conference will be republished in book form and the royalties paid to the University of Michigan for a future re-evaluation conference or similar purposes.

H. B. G. R.

PREVENTION OF DRY SOCKETS

HAROLD W. KROGH, D.D.S.

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THE dry socket is the most common complication of tooth extraction. Many attempts have been made to control this distressing sequel to extraction but few have met with more than limited or occasional success. The purpose of this paper is to report a marked reduction in the incidence of dry socket as a result of placing a tablet containing ½ grain of sulfanilamide and ½ grain of sulfathiazole in the alveolus immediately after extraction.

The use of sulfonamides in dental surgery is not new; in fact, the practice was the subject of a comprehensive review which appeared in 1942. The Council on Therapeutics of the American Dental Association authorized a study in which Ostrander and Hartman[1] analyzed the contents of seventy-two previously published reports. The conclusions reached were unfavorable to the use of sulfonamides in dental surgery chiefly because of four weaknesses in the studies reported. According to the reviewers these objections were: (1) lack of adequate controls; (2) use of subjective rather than objective criteria; (3) frequent combination of therapeutic agents; (4) insufficient number of cases.

The first objection was "lack of adequate controls." A series which furnishes an adequate control group was reported in 1937 by Krogh.[2] This report gave the incidence of dry socket following the removal of 6,403 teeth under procaine hydrochloride anesthesia from 2,110 patients over a six-year period. The percentages established in the 1937 report will be used as controls for those derived from the present study.

The second objection was "the use of subjective rather than objective criteria." In the 1937 report, a dry socket was defined as "a socket in which the blood clot disintegrates with the production of a foul odor, without pus formation, and accompanied by a severe neuralgia of the fifth cranial nerve, persisting over a period of days." The same criteria were used in the present series. The disintegration of the blood clot and the foul odor can certainly be determined objectively, and whereas pain must be regarded as a subjective symptom, the pain of a dry socket is so typical that it can usually be evaluated objectively. Inconsistency resulting from the opinions of a number of individuals is precluded, because all patients in both series were examined postoperatively by the same person.

The third objection was "frequent combination of therapeutic agents." By this is meant therapeutic agents other than the sulfonamides. No basis for such an objection exists in this series because only sulfonamides were employed. Sulfanilamide and sulfathiazole were employed in this study because of their

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different solubility rates; the former is quite soluble, whereas the latter goes into solution much more slowly. In addition this combination is more effective against a larger variety of bacteria than either single component. Immediately after every extraction, two antagonistic forces become operative: the first an effort of the body to organize the clot; the second, an effort of the oral bacteria, enzymes, or unknown factors to destroy it. If the clot does not become organized a dry socket may result. The rationale for the use of the sulfonamide drugs in sockets is their possible inhibitory effect on clot-destroying bacteria. The sulfonamides may be and are used in many forms and media in dentistry, but in these cases they were used only in the form of keystone-shaped tablets. These were used in preference to either powder or crystals because they are not washed out by the immediate postoperative bleeding. Neither do they cause trouble if they are included within the healing tissues as is sometimes the case with ointments.

The fourth objection was "insufficient number of cases." The 1937 report was based on the removal of 2,126 lower molars and premolars, the present report on the removal of 773 similar teeth. All cases in both series were consecutive and not selected. These numbers are sufficient to provide an adequate basis for an analysis which will be statistically valid.

This report is based on extractions carried out in a dental office during the entire year of 1944. Procaine hydrochloride was the anesthetic used in all cases. The only change from the 1937 technique was in the insertion of the tablet in the sockets: one tablet in premolar sockets and two in molar sockets. Three tablets were used in cases of partial uneruption when an inflammatory process was present. Only extractions of permanent lower molar and premolar teeth and roots were tabulated since it was in the sockets of these teeth that 95 per cent of the dry sockets developed in the 1937 series. Partially unerupted teeth are defined as those with their anatomic crowns only partially covered by hard or soft tissue or both. Unerupted teeth are those with their anatomic crowns completely covered with hard or soft tissues or both.

All patients in both series received the same postoperative instructions, namely: to apply an ice bag to the face for from four to six hours after operation, to avoid the use of mouthwashes until the next morning, to take a mild laxative at bedtime, and to use warm rinses of hypertonic saline solution several times daily for a few days following operation. Patients were directed to return on the second postoperative day and were observed until normal healing was assured, or until painful symptoms subsided.

The data presented here have been derived from a review of the cases in which operation was performed in 1944 under the stated conditions. In Tables I, II, and III, the term operation, as it is used, needs clarifying. An operation is an extraction of one or more teeth at a single sitting. The same patient may have several teeth extracted. The removal of several teeth at one sitting constitutes a single operation; at several sittings, a like number of operations. Thus, the number of operations is neither the same as the number of teeth removed nor the number of patients operated on.

Table I gives the general data regarding number of patients, sex distribution, number of operations, and number of teeth.

TABLE I

GENERAL DATA (ALL CASES)

PATIENTS		OPERATIONS	
Male	348	Male	379
Female	350	Female	378
Total	698	Total	757
TEETH EXTRACTED			
Erupted	850		
Unerupted	50		
Partially unerupted	17		
Total	917		

Each patient was urged to return at a definite time for postoperative inspection; nevertheless 131 patients (20.3 per cent) failed to do so. Table II gives the essential information concerning these rejected cases. This material was not included in the calculations.

TABLE II

GENERAL DATA (REJECTED CASES)

Patients	131	Teeth extracted	144
Operations	131	Erupted	143
Male	62	Partially unerupted	1
Female	69		

Table III gives the corrected general data.

TABLE III

CORRECTED GENERAL DATA

PATIENTS		OPERATIONS	
Male	286	Male	317
Female	281	Female	309
Total	567	Total	626
TEETH EXTRACTED			
Erupted	707	1st Premolar	97
Partially unerupted	49	2nd Premolar	120
		1st Molar	139
Unerupted	17	2nd Molar	186
		3rd Molar	165
Total	773		

It should be noted that in the 1937 series all procaine hydrochloride used was of 2 per cent strength, whereas, in the 1944 series, both 2 per cent and 4 per cent solutions were used. Procaine hydrochloride, 4 per cent, was used only in those cases (16 per cent) in which the teeth had been or were aching or there was evidence of an active inflammatory process in either the periapical, periodontal, or pulpal tissues (Table IV).

TABLE IV

TYPES OF NOVOCAIN USED

TYPE	%	CONC.	GM./C.C.
1937 Series			
Metz "E"	2	1-20,000	.00005
Metz "T"	2	1-50,000	.00002
Abbott Procain #2	2	1-25,000	.00004
Metz "K"	2	1-65,000	.000015
Cook "A"	2	1-50,000	.00002
Cobefrin-Cook	2	1-10,000	.0001
1944 Series			
Abbott	2	1-60,000	.000016
Oradent*	4	1-2,500	.0004

*Not accepted by Council on Therapeutics ADA.

Table V gives a comparison of the two series broken down into numbers of teeth according to their morphology, number of dry sockets, and percentage of dry sockets. It demonstrates the astonishing fact that not a single dry socket occurred in the 1944 series of erupted teeth. The chances of such a difference occurring as a result of random selection is something like 1 in 100 million.

TABLE V

INCIDENCE OF DRY SOCKETS

1937 SERIES				1944 SERIES		
	NUMBER	DRY SOCKETS		NUMBER	DRY SOCKETS	
LOWER TEETH	EXTRACTED	NO.	%	EXTRACTED	NO.	%
1st Premolar	241	5	2.2	97	0	0
2nd Premolar	371	8	2.2	120	0	0
1st Molar	453	19	4.2	139	0	0
2nd Molar	460	23	5.0	186	0	0
3rd Molar	376	13	3.5	165	0	0
Totals	1901	68	Av. 3.1	707	0	0

TABLE VI

ACHING AND ACUTELY ABSCESSED TEETH

1944 SERIES			
ACHING		ACUTE	
1st Premolar	9	1st Premolar	0
2nd Premolar	14	2nd Premolar	5
1st Molar	21	1st Molar	7
2nd Molar	36	2nd Molar	3
3rd Molar	17	3rd Molar	1
Total	97	Total	16

Table VI shows that 133 (16 per cent) of the erupted teeth were removed at a time not favorable for optimum healing. Despite this fact all sockets healed without unfavorable incident. Procaine hydrochloride, 4 per cent, was used in these cases. It may be possible that patients who were formerly denied extraction of erupted teeth until after the acute symptoms had subsided, can now be given relief by immediate removal of the offending teeth. This procedure may be extended to unerupted teeth if general anesthesia is used.

TABLE VII

INCIDENCE OF DRY SOCKETS
UNERUPTED LOWER THIRD MOLARS

	1937 SERIES			1944 SERIES		
IMPACTIONS	NO.	NO. DRY SOCKETS	%	NO.	NO. DRY SOCKETS	%
Complete	112	32	28.7	17	0*	0
Partial	113	30	26.5	49	0*	0
Totals	225	62	27.6 (av.)	66	0*	0

*See case reports.

Table VII shows that in the most difficult extractions, i.e., those of unerupted teeth, the reduction of incidence of dry socket associated with the use of sulfonamides is striking. The chances of such a difference occurring from the operation of random selection is about 1 in 5,000,000. There were no typical dry sockets in this group; however, there were six cases that required postoperative treatment before proper healing occurred; these will be discussed individually.

Miss K. N. returned with pus in the socket twenty-one days following the removal of an unerupted lower left third molar. A rubber dam drain was inserted distal to the second molar and kept in place for seven days. Healing from then on was uneventful.

Miss J. D. returned with a purulent exudate from the socket on the fourth day following the removal of an unerupted lower left third molar. A rubber dam drain was inserted in the socket and kept in place for four days. No further treatment was necessary.

In these two patients the clot disintegrated, but since neither the foul odor nor the neuralgia was present, the condition was not regarded as true dry socket. Production of pus is occasionally seen following the removal of unerupted teeth and usually is due to traumatized bone, which subsequently becomes necrotic. In neither of these two cases could necrotic bone nor any foreign body be demonstrated.

Mrs. A. A. complained of pain in her ear on the eighth day following the removal of an unerupted lower right third molar. A piece of one-fourth inch gauze, about one inch long, saturated with eugenol, was placed in the socket. The pain subsided quickly and the dressing was removed the following day. No further treatment was needed.

Mr. F. C. had had a partially unerupted lower left third molar removed. Pain in the ear developed on the fifth postoperative day. A eugenol dressing inserted in the socket and left for three days gave complete relief.

Mrs. B. E. K. needed relief from pain on the sixteenth postoperative day following a difficult extraction of the unerupted lower left third molar. A eugenol dressing was inserted and changed five times in the next eleven days, after which there was no further pain.

These three patients each had a neuralgia which was not quite typical of that associated with dry socket, and since the foul odor and the clot disintegration were absent, the condition was not so considered.

Miss H. B. was the only individual of this series in whom a generalized reaction occurred. A minute macular eruption was apparent on the extremities, and according to the patient, the entire body was covered on the fourth postoperative day following the removal

of the unerupted lower right third molar. The remains of the sulfonamide tablets were thoroughly washed from the socket. Three days later a few lesions appeared on the oral mucous membranes although the skin had already stopped itching. It was subsequently brought out that this patient was sensitive to sulfathiazole. The socket healed without evidencing any of the symptoms of dry socket.

DISCUSSION

Data have been presented on the incidence of dry socket with and without the use of sulfonamides. The tabulations suggest that the sulfonamide tablets were beneficial and do not delay healing, because they markedly reduce the greatest cause of delayed socket healing, namely, the dry socket. No clinical evidence of foreign body reaction has been seen, and the presence of the tablets seems to be compatible with the growth of granulation tissue. Occasionally, parts of tablets are seen in sockets a week or so postoperatively, but since the patient is not having pain and there is no putrefaction in the socket, their presence is not considered objectionable. As the new tissue grows, the remaining drug is either expelled, dissolved, or absorbed. It has not been necessary to resort to operation to remove these fragments in any case.

SUMMARY

The experience gained in removing 773 lower molars and premolars during 1944 has been compared to that of removing 2,126 morphologically similar teeth over a period of six years, reported in 1937. The only difference in technique, except the change in procaine hydrochloride used, is that sulfonamide tablets were placed in the sockets of extracted lower molars and premolars in the 1944 series. The results are highly favorable to the employment of sulfonamides in such procedures.

No typical dry socket developed in sixty-six sockets of unerupted teeth, as compared to sixty-two (27.6 per cent) in 225 unerupted teeth in the control series. The chances of such a difference occurring as a result of a random selection is about 1 in 5 million.

No dry sockets developed in 707 sockets of erupted teeth, as compared to 68 (3.1 per cent) in 1901 erupted teeth in the control series. The chances of such a difference occurring as a result of random selection is about 1 in 100 million.

The author gratefully acknowledges the assistance rendered by Paul Bruyere, M. D., Chief of the Statistical Division Army Institute of Pathology, and Frank M. Weida, Ph. D., Professor of Statistics, George Washington University, in the statistical analysis of these data.

REFERENCES

1. Ostrander, F. D., and Hartman, F. W.: Local Use of Sulfonamides, *J. A. D. A.* **29**: 1051, 1942.
2. Krogh, Harold W.: Incidence of Dry Socket, *J. A. D. A.* and *Dental Cosmos* **24**: 1829, 1937.

ACUTE VITAMIN C DEFICIENCY AND PERIODONTAL DISEASE

I. The Periodontal Tissues of the Guinea Pig in Acute Vitamin C Deficiency

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THE effects of vitamin C deficiency have been shown to result from a failure in the formation and maintenance of intercellular substances in tissues of mesenchymal origin.[1] Changes in the periodontal tissues in vitamin C deficiency have been described and a relationship between such changes and periodontal disease has been suggested.[2-7] It was felt, however, that by bringing together the findings on the gingivae and underlying structures in one study, the exact manner in which microscopic tissue changes in acute vitamin C deficiency are related to the initiation and progress of periodontal disease could be established. This was attempted in the following experiment.

EXPERIMENTAL PROCEDURE

Twenty-five young adult guinea pigs weighing from 200 to 300 grams were used as experimental animals. Sixteen of the animals were placed on a vitamin C-free diet. Nine animals which served as controls received the same diet supplemented by ascorbic acid in a dosage of 1 mg. per 100 Gm. of body weight. The animals were sacrificed after thirty-five days. The mandible, maxilla, and knee joint were decalcified in an equal mixture of formic acid and sodium citrate, embedded in paraffin, and prepared in serial section for microscopic study.

OBSERVATIONS

Epidiaphyseal Junction—Distal End of the Femur

Control Animals

The epiphyseal cartilage cells were arranged in regular columns (Fig. 1). The individual cells presented uniform differences and appeared flattened, hypertrophied, degenerated, and fragmented as the distance from the epiphyseal line increased. Elongated trabeculae of bone extended from the cartilage into the diaphyseal region. Osteoblasts adjacent to pale-staining osteoid matrix were seen along the margins of the bone trabeculae (Fig. 2).

Vitamin C-Deficient Animals

The appearance of the cartilage and bone was characteristic of acute vitamin C deficiency. The regular columnar arrangement and appearance of the cartilage cells were distorted (Fig. 3). The bone trabeculae were thin with irregularly eroded margins. The osteocyte lacunae were enlarged and, in many instances, empty. There were no osteoblasts or new bone formation (Fig. 4).

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Fig. 1.—Control animal. Epidiaphyseal junction, distal end of femur showing regular arrangement of cartilage, cartilage cell changes, and contiguous bone trabeculae. (Hematoxylin and eosin; orig. mag. ×96.)

Fig. 2.

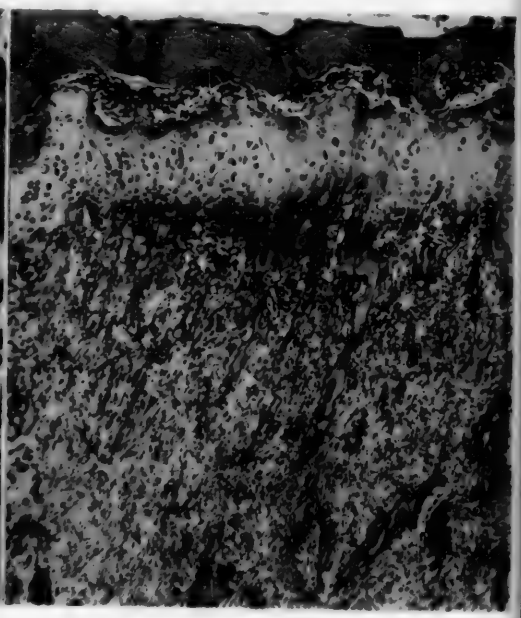

Fig. 3.

Fig. 2.—Control animal. Detail of Fig. 1. Bone trabecula with adjacent osteoblasts and marginal osteoid matrix. (Hematoxylin and eosin; orig. mag. ×280.)

Fig. 3.—Vitamin C-deficient animal. Epidiaphyseal junction, distal end of femur showing irregular arrangement and distorted appearance of cartilage cells. Note thin bone trabeculae. (Hematoxylin and eosin; orig. mag. ×96.)

Gingival Sulcus and Gingival Papilla

Incisor Region

Control Animals

The gingival sulcus appeared as a shallow V-shaped crevice, the lateral wall of which was lined with stratified squamous epithelium. The marginal gingiva was covered by markedly keratinized stratified squamous epithelium which was continuous with the epithelial lining of the sulcus. The underlying connective tissue core of the marginal gingiva was comprised of vertical collagen fibers interspersed with elliptical fibrocytes and a moderate distribution of blood vessels.

Vitamin C-Deficient Animals

The gingival sulcus and epithelium covering the marginal gingiva were similar to those of the control animals. The connective tissue core of the marginal gingiva, however, was edematous, with small zones of hemorrhage. The collagen was clumped in bundles which were separated by edema. The outlines of the individual collagen fibrils were not sharply demarcated from each other. The fibrocytes appeared shrunken and hyperchromic.

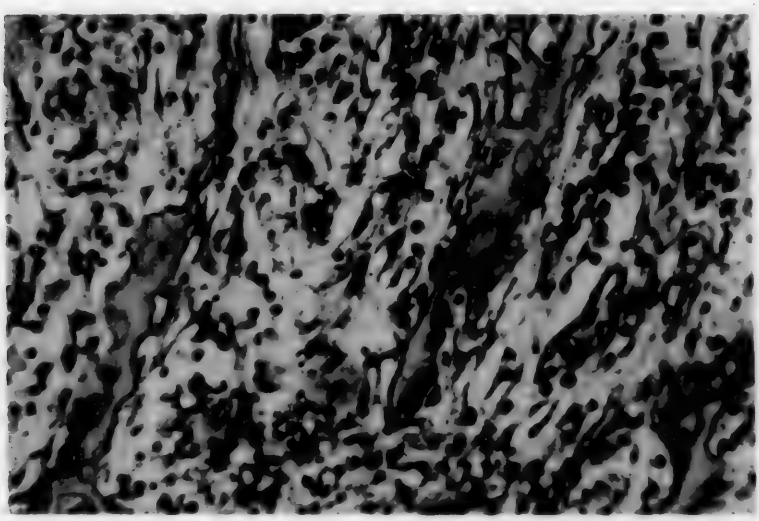

Fig. 4.—Vitamin C-deficient animal. Detail of Fig. 3, showing bone trabeculae with irregularly eroded margins and enlarged osteocyte lacunae. Note absence of osteoblasts or osteoid matrix. (Hematoxylin and eosin; orig. mag. ×24.)

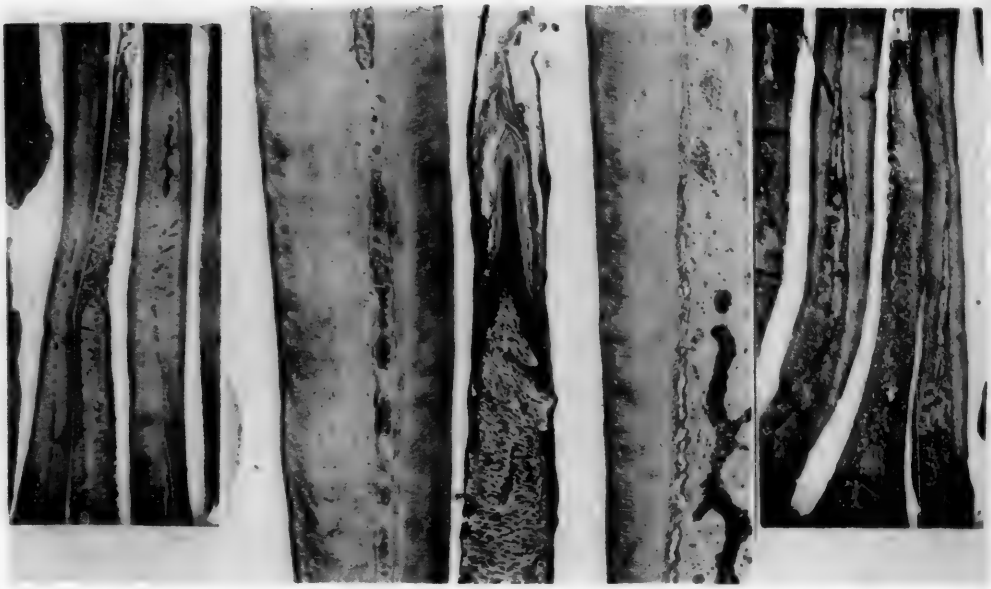

Fig. 5. Fig. 6. Fig. 7.

Fig. 5.—Control animal. Molar area, showing interdental gingival papilla and underlying alveolar bone. (Hematoxylin and eosin; orig. mag. ×240.)

Fig. 6.—Control animal. Detail of Fig. 5, showing gingival sulci and interdental gingival papilla. Note debris and degenerated cells in sulcus. (Hematoxylin and eosin; orig. mag. ×108.)

Fig. 7.—Vitamin C-deficient animal. Molar area, showing interdental gingival papilla, underlying alveolar bone, and adjacent teeth. (Hematoxylin and eosin; orig. mag. ×24.)

Molar Area

Control Animals

The gingival sulcus appeared as a V-shaped space containing a scattering of polymorphonuclear leucocytes, desquamated keratinized strands, and food particles (Figs. 5 and 6). One wall of the sulcus was formed by a layer of cementum of varied thickness; the other, by the superficial keratinized surface of the interdental gingival papilla. The interdental gingival papilla consisted of a comparatively thin core of collagenous connective tissue and a thick covering of markedly keratinized stratified squamous epithelium. A few leucocytes were occasionally seen in the connective tissue beneath the bottom of the sulcus.

Vitamin C-Deficient Animals

The gingival sulcus in the vitamin C-deficient animals appeared similar to that in the control animals. In many instances the epithelial attachment extended as elongated projections along the cementum for sufficient distance to be noted, but not enough to alter the sulcus depth significantly (Figs. 7 and 8). The connective tissue core of the interdental papilla presented edema, collagen degeneration, and small zones of hemorrhage. A few leucocytes were occasionally seen in the connective tissue beneath the bottom of the sulcus.

Fig. 8.

Fig. 9.

Fig. 8.—Vitamin C-deficient animal. Detail of Fig. 7, showing gingival sulci and interdental gingival papilla. Note edema and collagen degeneration in the underlying connective tissue. (Hematoxylin and eosin; orig. mag. ×108.)

Fig. 9.—Control animal. Incisor area, lingual aspect, showing division of periodontal fibers into a vertical group (adjacent to tooth) and a horizontal group (adjacent to bone). Note bone formation along lingual bony plate. (Hematoxylin and eosin; orig. mag. ×96.)

The Periodontal Membrane

Incisor Area

Control Animals

The periodontal membrane could be divided into two portions based on the distribution and arrangement of the collagen fibers. The portion adjacent to the tooth was denser than that near the bone and consisted of closely packed collagen fibers arranged parallel with the tooth (Fig. 9). A layer of polyhedral cells was seen adjacent to the cementum. In the periodontal membrane adjoining the bone the fibers were fewer in number and interspersed among numerous fibrocytes. Capillaries were also observed more frequently in this area.

Vitamin C-Deficient Animals

Although the entire periodontal membrane was altered, the severest change was observed in the portion adjacent to the alveolar bone. Throughout the length of the periodontal membrane the portion adjoining the bone was almost completely devoid of well-formed collagenous fibers (Figs. 10 and 11). Instead, the space was filled with hemorrhage and detached remnants of degenerated collagen. The periodontal fibers adjacent to the tooth were edematous. The clear-cut fibrillar outlines observed in the collagen of control animals was obscured. The fibers blended with each other.

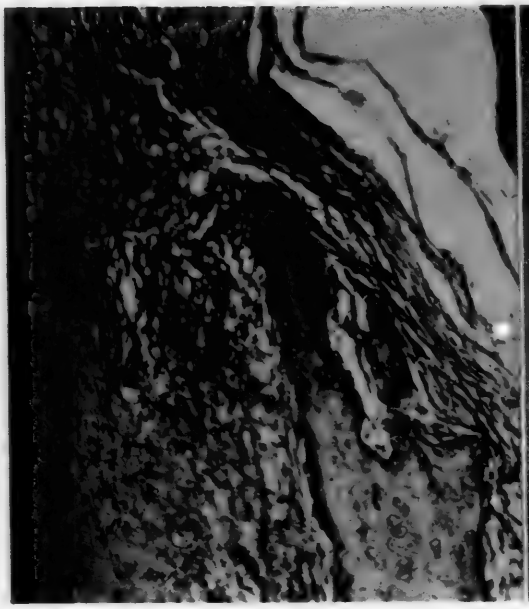

Fig. 10.

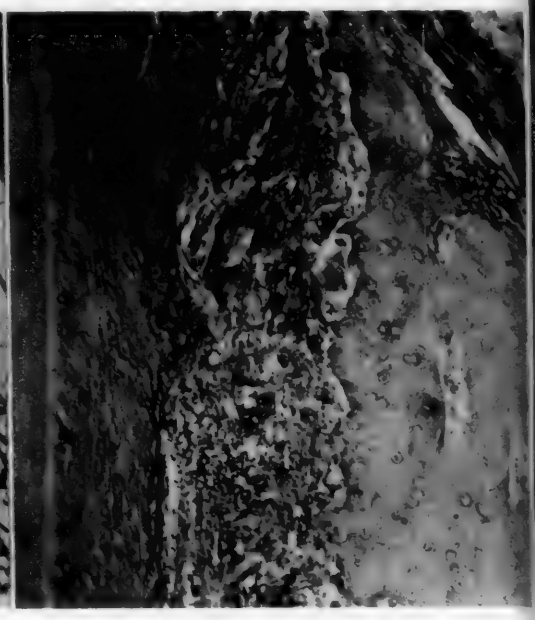

Fig. 11.

Fig. 10.—Vitamin C-deficient animal. Incisor area lingual aspect, showing edema and degeneration of collagen and absence of bone formation. (Hematoxylin and eosin; orig. mag. ×108.)

Fig. 11.—Vitamin C-deficient animal. Incisor area, lingual aspect, showing difference in the appearance of the periodontal membrane. Adjacent to the bone (right) there is hemorrhage and marked degeneration of the collagen. Adjacent to the tooth (left) the collagen presents comparatively slight degenerative change. Note eroded bone margin and break in the continuity of the collagen fibers and bone matrix. (Mallory's connective tissue stain; orig. mag. ×150.)

Molar Area

Control Animals

The periodontal membrane surrounding the alveolar bone consisted of dense collagen fibers with numerous elliptical fibrocytes and blood vessels (Fig. 12). There was a suggested division of the fibers into vertical and horizontal portions which was less pronounced than that observed in the incisor region. In some areas capillaries extended from the periodontal membrane into vessel channels of the interdental bone.

Vitamin C-Deficient Animals

The periodontal membrane was edematous and hemorrhagic throughout (Fig. 13). Severest changes were observed adjacent to the bone. The collagen presented degeneration which varied in degree from edema of individual fibrils to fragmentation and dissolution. For the most part, the continuity of the periodontal fibers and alveolar bone was destroyed.

Fig. 12. Fig. 13.

Fig. 12.—Control animal. Interdental bony septum, molar, showing extensions from periodontal membrane into bone. Note osteoblasts along the mesial aspect of bone (left) and osteoclasts along the distal (right). (Hematoxylin and eosin; orig. mag. ×120.)

Fig. 13.—Vitamin C-deficient animal. Interdental bony septum, molar area, showing edema and degeneration of periodontal membrane fibers. The bone margins are eroded throughout. The vessel channels are enlarged. There is no evidence of new bone formation. (Hematoxylin and eosin, magnification ×108.)

Alveolar Bone

Incisor Area

Control Animals

The alveolar bone presented a thin layer of pale pink matrix along the margin adjacent to the periodontal membrane and along the gingival crest (Fig. 9). The collagenous fibers of the periodontal membrane appeared continuous with the marginal bone matrix. At the margin of the bone and periodontal membrane the collagen progressively lost its fibrillar nature and appeared granular so that it was not possible to discern where the collagen terminated and where bone matrix began. Osteoblasts in apposition with the bone substance and in some areas partially embedded in it were seen interspersed between the collagenous fibers.

Vitamin C-Deficient Animals

The surface of alveolar bone adjoining the periodontal membrane was eroded (Figs. 10 and 11). Interspersed along the eroded surface an occasional enlarged concavity with an enclosed multinuclear osteoclast was seen. There were no osteoblasts or newly formed pale pink osteoid matrix. The continuity of the collagen fibers and bone matrix was destroyed. The osteocyte lacunae near the eroded bone margin were enlarged, with pyknotic nuclei. In many animals a strip of degenerated collagenous prebony matrix was seen adjacent to the labial bony plate (Fig. 14). Contained within the degenerated collagen matrix were many shrunken hyperchromic cells.

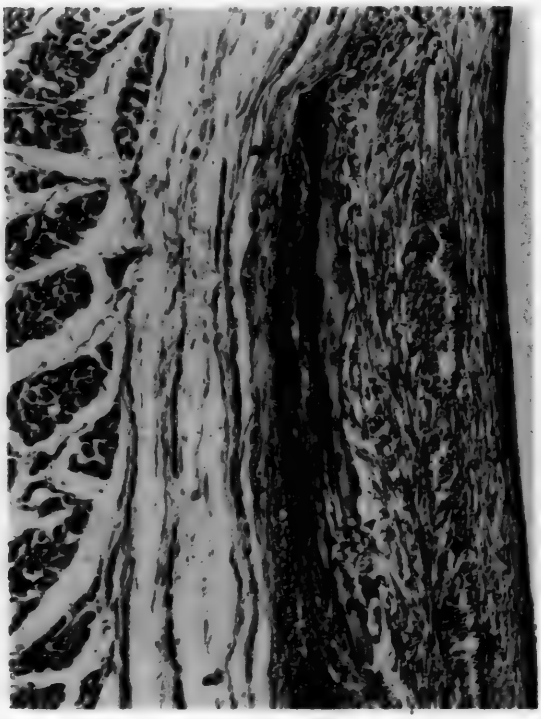

Fig. 14.—Vitamin C-deficient animal. Incisor region, labial aspect, showing strip of degenerated collagenous prebony matrix adjacent to alveolar bone. (Hematoxylin and eosin, orig. magnification ×150.)

Molar Area

Interdental Septum

Control Animals

Along the mesial aspect and tip, the interdental alveolar bone was lined with a beadlike layer of cuboidal osteoblasts (Fig. 12). In some areas a thin osteoid matrix separated the osteoblasts from the underlying bone. The distal margin of the interdental septum was formed by shallow continuous concavities in which pyriform multinuclear osteoclasts were seen. The entire interdental septum was traversed by numerous vessel channels.

Vitamin C-Deficient Animals

The interdental alveolar bone was markedly osteoporotic (Fig. 13). Along the mesial and distal aspects and within the bony septum, large eroded concavities containing numerous large multinuclear osteoclasts were seen. There was no evidence of osteoblastic activity. The vessel channels were enlarged and presented eroded bony margins.

Bone Between the Root Apices and the Border of the Mandible

Control Animals

With the exception of occasional small indentations the bone margins of the medullary spaces were smooth and lined with small cuboidal endosteal cells (Fig. 15). A thin layer of pale pink bone matrix separated these cells from the underlying bone a long approximately one-half of the total endosteal surface.

Vitamin C-Deficient Animals

The medullary spaces were enlarged and lined with thin, flattened endosteal cells (Fig. 16). For the most part the endosteal bone margins were formed by small, irregular, shallow indentations. Occasional multinuclear osteoclasts were seen in large, bony, marginal concavities. With the exception of a few smaller scattered zones of pale pink matrix, there was no evidence of endosteal bone formation.

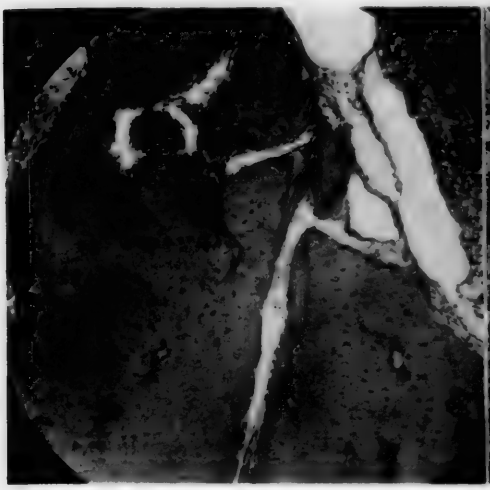

Fig. 15.

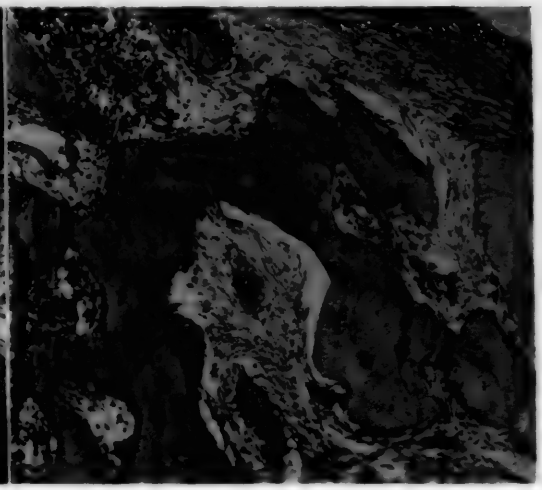

Fig. 16.

Fig. 15.—Control animal. Alveolar bone between root apices and lower border of the mandible showing vessel channels and medullary spaces with smooth margins, osteoblasts, and areas of new bone formation. (Hematoxylin and eosin, orig. magnification ×120.)

Fig. 16.—Vitamin C-deficient animal. Alveolar bone between root apices and lower border of the mandible showing enlarged medullary spaces with eroded margins and absence of new bone formation. (Hematoxylin and eosin, orig. magnification ×140.)

Fig. 17.

Fig. 18.

Fig. 17.—Control animal. Distal surface of the molar showing deepened gingival sulcus attached just below cemento-enamel junction. (Hematoxylin and eosin, orig. magnification ×45.)

Fig. 18.—Control animal. Detail of Fig. 17. Bottom of periodontal pocket, periodontal membrane, and alveolar bone. Note well-formed collagen in periodontal membrane. There is new bone formation along the alveolar bone margin. (Hematoxylin and eosin, orig. magnification ×126.)

Pocket Formation

Control Animals

Deepening of the gingival sulcus was observed only at the mesial surface of the last molar. The base of the pocket was attached slightly below the cemento-enamel junction (Fig. 17). Amorphous food and tissue debris were contained within the periodontal pockets. Within the debris and within the superficial epithelial layers of the adjacent epithelium were a few necrotic polymorphonuclear leucocytes. The connective tissue underlying the base of the pocket consisted of a dense collagenous stroma containing many elliptical fibrocytes (Fig. 18). The margin of the alveolar bone was lateral to the base of the pocket and slightly below it. A thin, pale pink osteoid matrix lined with osteoblasts formed the superficial bony margin.

Vitamin C-Deficient Animals

Periodontal pocket formation was observed at only the mesial surface of the first molar and distal surface of the last molar. In general, the pockets were deeper than those observed in similar areas in the control animals and extended for a greater distance along the cementum (Fig. 19). The connective tissue underlying the base of the pocket was edematous and consisted for the most part of loosely arranged, markedly degenerated collagenous fibers and scattered shrunken fibrocytes (Fig. 20). The pockets contained amorphous debris interspersed with necrotic polymorphonuclear leucocytes. These cells were also contained within the superficial layers of the epithelium lining the pocket. The depth of the pockets along the cementum was such that a considerable portion of the pocket wall extended below the superficial border of the adjoining alveolar bone. The alveolar bone presented an irregularly eroded margin. Polyhedral hyperchromic cells were observed within lacunar indentations of the bony margin. There was no evidence of new bone formation.

Fig. 19.

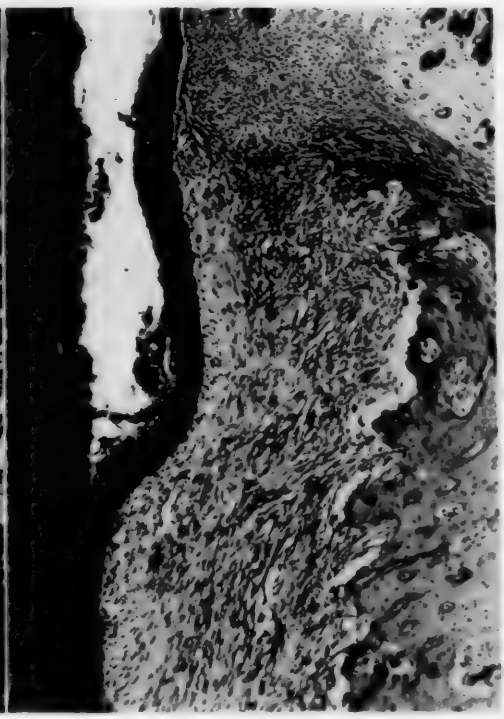

Fig. 20.

Fig. 19.—Vitamin C-deficient animal. Distal surface of molar showing deepened gingival sulcus attached along cementum at a considerable distance from the cemento-enamel junction, below the level of the alveolar bone. (Hematoxylin and eosin, orig. magnification ×60.)

Fig. 20.—Vitamin C-deficient animal. Detail of Fig. 19. Bottom of periodontal pocket, periodontal membrane, and alveolar bone showing bottom of pocket below the level of the alveolar bone margin. The periodontal membrane is edematous and degenerated throughout. The adjacent bone surface is eroded. There is no evidence of new bone formation. (Hematoxylin and eosin, orig. magnification ×120.)

DISCUSSION

The existence of acute vitamin C deficiency in individual animals was established on the basis of microscopic changes in the epidiaphyseal junction in the distal end of the femur. Related alterations in the periodontal tissues which also resulted from acute vitamin C deficiency were noted. It is to a consideration of the latter alterations and their possible significance in the over-all problem of periodontal disease that this discussion is directed.

Gingival inflammation was not a prominent finding in either the vitamin C-deficient or control animals. When gingival inflammation occurred in either group of animals it was associated with food remnants and debris in the gingival sulcus. In both groups of animals the inflammation was comparable and was of slight severity. There was no increase in the incidence of gingival inflammation in the vitamin C-deficient animals. It is significant that notwithstanding the fact that marked changes such as edema, collagen degeneration, and hemorrhage do alter the connective tissue core of the marginal gingivae in acute vitamin C deficiency, the deficiency of itself does not cause marginal gingivitis.

Pocket formation was observed in both the vitamin C-deficient and control animals only along the mesial surface of the first molar and distal surface the last molar. There was no other appreciable pocket formation in the control or vitamin C-deficient animals. Neither of the involved areas was protected by the contact of an approximating tooth. These facts suggest that initiation of pocket formation is not especially associated with acute vitamin C deficiency and that the pockets observed in both the control and vitamin C-deficient animals were formed as the result of forcible food impaction. In neither group of animals was there a pronounced inflammatory involvement associated with pocket formation.

The depth of the periodontal pockets was greater in the vitamin C-deficient animals than in the controls. In the vitamin C-deficient group, the base of the pocket was well along the cementum and below the level of the adjoining alveolar bone. In the control group, the base of the pocket was slightly below the cemento-enamel junction and slightly above the marginal level of the alveolar bone. The bone adjoining the pocket in the vitamin C-deficient animals was osteoporotic and the periodontal membrane was edematous, hemorrhagic, and degenerated. In the control animals new bone formation was observed along the bone margin in relation to the base of the pocket, and the periodontal membrane was essentially unaltered.

It appears, therefore, that when pocket formation does occur in animals with acute vitamin C deficiency associated with a local factor such as food impaction, it is of greater depth than that which occurs under comparable local conditions in control animals. Degenerative changes in the periodontal membrane which result from acute vitamin C deficiency are responsible for this

exaggerated pocket depth. By reducing the barrier formed by the dense collagen bundles normally found in this area, the degeneration of collagen in acute vitamin C deficiency facilitates migration of the epithelial attachment along the cementum. Although of themselves not sufficient to initiate pocket formation, the periodontal membrane changes in acute vitamin C deficiency are significant regulating factors which permit extensive pocket depth in the presence of an added local stimulus.

Edema, hemorrhage, and pronounced collagen degeneration were observed in the periodontal membrane in the vitamin C-deficient animals. It is noteworthy that these changes were, for the most part, limited to the periodontal membrane adjoining the alveolar bone. The vertical collagen fibers adjoining the teeth presented comparatively slight edematous changes. Explanation for this difference in response in different portions of the periodontal membrane to acute vitamin C deficiency may be derived from analysis of the structure of the periodontal membrane. The collagen bundles adjoining the tooth are arranged vertically to accommodate the continuous tooth eruption. The collagen attached to the bone is arranged perpendicular to the alveolar border. It is not unlikely that the severity of the changes observed in acute vitamin C deficiency in the portion of the periodontal membrane adjoining the alveolar bone results from the combined effects of the deficiency and the functional stresses in the area.

In general, the alveolar bone in the vitamin C-deficient animals was osteoporotic. The bone in relation to the teeth presented essentially no osteoblastic activity or new bone. In the bone between the root apices and border of the mandible slight evidence of the endosteal bone formation was observed. The vessel channels and medullary spaces were enlarged and eroded throughout.

The changes observed in the periodontal membrane and bone in this study have been mentioned in the literature to which reference[2-7] has been made, and it has been pointed out that by weakening the support of the teeth these changes induce tooth mobility. It has also been suggested that periodontal destruction and tooth mobility in vitamin C deficiency are consistent with what, in human beings, has been termed diffuse alveolar atrophy or generalized periodontoclasia.[8] This opinion is supported by the findings in the present experiment.

Although the destruction of supporting tissues in vitamin C deficiency is striking, it cannot be inferred that in all instances of generalized periodontoclasia vitamin C deficiency is the causative factor. The production of generalized periodontoclasia in experimental animals[9-11] under other systemic conditions discourages such inference. The occurrence of generalized periodontoclasia cannot be considered pathognomonic of vitamin C deficiency.

It is noteworthy that destructive changes occur in the periodontal membrane and alveolar bone in acute vitamin C deficiency in the absence of pocket forma-

tion. It has been pointed out that, of itself, acute vitamin C deficiency apparently does not initiate pocket formation. Therefore, when pocket formation and destruction of the underlying tissues occur together in acute vitamin C deficiency, there is indicated the presence of a complicating local factor of sufficient severity to initiate the former change.

SUMMARY AND CONCLUSIONS

Using twenty-five young adult guinea pigs over a period of thirty-five days a controlled experiment was conducted to determine the manner in which the changes which occur in the gingivae and supporting tissues in acute vitamin C deficiency are related to the initiation and progress of periodontal disease.

The following are the pertinent facts which evolved from the microscopic findings:

In the periodontal membrane and alveolar bone, acute vitamin C deficiency is responsible for destructive changes in the absence of gingival inflammation or pocket formation. Because these changes result in a loss of tooth-supporting tissues throughout the jaws they produce a condition which may be described as generalized periodontoclasia or diffuse alveolar atrophy.

Although acute vitamin C deficiency causes the connective tissue of the marginal gingivae to be altered by edema, collagen degeneration, and hemorrhage, the deficiency itself is not responsible for the initiation of, or an increase in, the incidence of marginal gingivitis.

Pocket formation is not initiated by acute vitamin C deficiency; a complicating local factor is required before pocket formation occurs. However, when pocket formation does occur in acute vitamin C deficiency it is of greater depth than that which occurs normally under comparable local conditions. The concomitant occurrence of both pocket formation and destruction of underlying tissues in acute vitamin C deficiency is not attributable to the deficiency alone but indicates the presence of a complicating local factor.

The technical assistance of Mrs. Jane Zimmer in preparing the material for this study is acknowledged with gratitude by the author.

REFERENCES

1. Wolbach, S. B., and Bessey, O. A.: Tissue Changes in Vitamin Deficiencies, *Physiol. Rev.* **22**: 233, 1942.
2. Wolbach, S. B., and Howe, P.: Intercellular Substances in Experimental Scorbutus, *Arch. Path.* **1**: 1, 1926.
3. Boyle, P. E., Bessey, O. A., and Wolbach, S. B.: Experimental Production of the Diffuse Alveolar Bone Atrophy. Type of Periodontal Disease by Diets Deficient in Ascorbic Acid, *J. A. D. A.* **24**: 1768, 1937.
4. Hojer, A., and Westin, G.: Jaws and Teeth in Scorbutic Guinea Pigs, *Dent. Cosmos* **67**: 1, 1925.
5. McLean, D. L., Sheppard, M., and McHenry, E. W.: Tissue Changes in Ascorbic Acid Deficiency Guinea Pigs, *Brit. J. Exper. Path.* **40**: 451, 1939.
6. Hojer, J. A.: Studies in Scurvy, *Acta Paediat.* **3**: 119, 1924.

7. Boyle, P. E., Bessey, O. A., and Wolbach, S. B.: Experimental Alveolar Bone Atrophy Produced by Ascorbic Acid Deficiency and Its Relation to Pyorrhea Alveolaris, *Proc. Soc. Exper. Biol. & Med.* **26**: 733, 1937.
8. Boyle, P. E.: Dietary Deficiencies as a Factor in the Etiology of Diffuse Alveolar Atrophy, *J. A. D. A.* **25**: 1436, 1938.
9. Becks, H., and Weber, M.: Influence of Diet on Bone System With Special Reference to the Alveolar Process and Labyrinthine Capsule, *J. A. D. A.* **18**: 197, 1931.
10. Becks, H., and Morgan, A.: The Effects of Deficiencies of the Filtrate Fraction of the Vitamin B. Complex and Nicotinic Acid on the Teeth and Oral Structures, *J. Periodontal* **13**: 18, 1942.
11. Glickman, I.: The Periodontal Structures in Experimental Diabetes, *New York J. Dent.* **16**: 226, 1946.

DETERMINATION OF THE APPROXIMATE VOLUMES OF MEDICATION USED IN ENDODONTIC TREATMENT

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THE most important procedure in endodontic treatment is the complete removal of infected tissue from the pulp chamber and root canal. This is in accordance with one of the accepted principles of surgery, débridement of a wound. This procedure is essential to the destruction of the remaining infectious organisms. Where the natural vascular defenses can operate, this problem is relatively simplified. In the infected root canal, however, where the natural defenses of the blood stream have reduced ability to function, the organisms remaining after débridement must be destroyed with medication.

It is valuable to determine how much medication can be sealed into a tooth prepared for root canal therapy, or in the case of antibiotics, how many units can be sealed into a root canal undergoing endodontic treatment. Empiric statements have been made concerning the capacity of a root canal and regarding the number of units of an antibiotic that may be sealed in a root canal. Shaw, Sprawson, and May[5] used a solution of penicillin containing 20,000 units per c.c. and estimated that only 50 units of penicillin could be sealed in a root canal at each treatment. Buchbinder[2] stated that "5,000 units of penicillin may easily be sealed in the average anterior root canal" when a solution containing 100,000 units of penicillin is used. Bender[1] estimated that the volume of a root canal is one-fifth c.c. or less, and that from 4,000 to 5,000 units of penicillin may be sealed in a root canal if 100,000 units per c.c. of solvent are employed. These estimates have been mere guesses. An attempt is made to elucidate this problem and to consider the relationship of the size of the tooth to the volume of medication that could be sealed in the contained root canal.

PROCEDURE

At least ten specimens of each type of extracted human tooth, except third molars, were used. In the anterior teeth, access to the root canal was gained by grinding through the cingulum area with a round bur. The contents of the pulp chamber and root canal were then removed and the canal was enlarged with reamers and files to a No. 5 or 6 instrument. Posterior teeth were similarly treated, except that the approach to the pulp chamber was made from the occlusal surface.

In the maxillary molar teeth the lingual root canal was enlarged to a No. 5 or 6 file, and the buccal root canals to at least a No. 4 file. In the mandibular molar teeth the distal root canals were enlarged to a No. 5 or 6 file and the mesial canals to a No. 4 instrument.

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Mercury was then placed in the pulp chamber of the tooth to be studied.* Beeswax, used as a plunger, was then applied until droplets of mercury emerged from the root apex. A small piece of beeswax was then placed over the apex, or apices in the case of multi-rooted teeth, and the tooth was then studied with the fluoroscope.

The purpose of the fluoroscope examination was to determine whether or not the root canals were completely filled, and also to make certain that the volume being occupied by the mercury was not greater than that occupied by the medicaments used in endodontic treatment.

In anterior teeth the space occupied by the medicament would consist of the entire root canal and that part of the pulp chamber not being used in sealing the medication. In the posterior teeth, all of the root canals and not more than one-third of the pulp chamber were filled with mercury. This, we believe, is the maximum volume of medication that can be sealed in a posterior tooth when a double seal is used. The double seal consists of a layer of gutta percha covered with cement.

If, after the fluoroscopic examination more than the specified quantity of mercury was present, pressure was applied with the beeswax plunger to force the excess mercury out through the apical foramen.

The mercury was then removed from the tooth and weighed on an analytical balance. After the mass of mercury had been determined the volume was calculated by using the method of Thiesen and Scheel.[4]

FINDINGS

The values obtained, as well as the measurements of the teeth used in this investigation, are shown in Tables I and II. The average values as well as the maximum and minimum values obtained are given so that the limits of variation may be seen.

TABLE I
MAXILLARY TEETH

	TOOTH						
	INCISOR FIRST	INCISOR SECOND	CANINE	PREMOLAR FIRST	PREMOLAR SECOND	MOLAR FIRST	MOLAR SECOND
Volume in c.c.							
Average	0.0163	0.0136	0.0230	0.0238	0.0159	0.0399	0.0346
Maximum	0.0222	0.0182	0.0281	0.0364	0.0260	0.0672	0.0495
Minimum	0.0128	0.0109	0.0143	0.0109	0.0093	0.0266	0.0259
Length in mm.							
Average	22.8	22.5	26.5	20.9	21.8	20.6	20.0
Maximum	25.0	24.0	29.0	23.5	23.0	22.0	23.0
Minimum	20.5	20.0	25.0	18.0	18.5	18.5	18.5

DISCUSSION

If we refer to the tables presented here, it is quite striking to note the minute volumes of drugs with which the endodontist must work. It is, therefore, most important to enlarge the canals adequately while removing the pulp debris. This will not only help to reduce the number of bacteria in the root

*As suggested by Dr. Arthur B. Gabel.

TABLE II
MANDIBULAR TEETH

	TOOTH				
	INCISOR*	CANINE	PREMOLAR*	MOLAR FIRST	MOLAR SECOND
Volume in c.c.					
Average	0.0088	0.0133	0.0156	0.0412	0.0325
Maximum	0.0180	0.0150	0.0192	0.0546	0.0460
Minimum	0.0052	0.0088	0.0126	0.0302	0.0207
Length in mm.					
Average	21.2	23.9	21.4	21.8	18.8
Maximum	23.0	25.0	23.0	24.0	22.0
Minimum	19.0	21.0	21.0	21.0	18.0

*Because there is relatively no difference in size between lower first incisors and lower second incisors, and the lower first and second premolars, the values for first incisors and first premolars only are given.

canal mechanically, but will also allow a greater volume of medication to be used for destroying the remaining bacteria.

The tables give the approximate volumes of medication that can be sealed into a tooth prepared for endodontic treatment. From these volumes the number of units of penicillin, or any other antibiotic that can be sealed in a root canal, can be computed, when the number of units per cubic centimeter is known. The number of units per cubic centimeter, times the average value given in the tables for the root canal in question, will give the approximate number of units which can be sealed into a tooth.

The average measurements of the teeth, as shown in the tables, compare very closely with those given by Churchill.[3] It is interesting to note that occasionally a shorter tooth will have a greater volume than a longer tooth.

In practice, the values given for the maxillary teeth may be slightly less than those indicated, since an absorbent point is usually sealed in each canal, in order to carry the medication to the apical area. However, since the absorbent point becomes saturated with the medicament used, it does not displace its own volume.

SUMMARY

1. The amount of medication that can be sealed in the root canals of various teeth is presented.

2. A method is given for determining the approximate number of units of an antibiotic that may be sealed in a pulpless tooth under endodontic treatment.

The author wishes to express his appreciation to Dr. Louis I. Grossman for suggestions in carrying out this study.

REFERENCES

1. Bender, I. B.: *J. A. D. A.* **34**: 99, 1947.
2. Buchbinder, M. J.: *J. D. Res.* **25**: 13, 1946.
3. Churchill, H. R.: Human Odontography and Histology, Philadelphia, 1932, Lea & Febiger, pp. 58-120.
4. Handbook of Chemistry and Physics, ed. 22, 1937-1938, Chemical Rubber Publishing Co., p. 1228.
5. Shaw, F. E., Sprawson, E., and May, H. B.: *Brit. M. J.* **1**: 551, 1945.

THE EVANSTON DENTAL CARIES STUDY

I. Determination of Fluorine in Foods, Bones, and Teeth

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THIS report is concerned primarily with the preliminary laboratory findings of the Dental Caries Study, Evanston, Illinois. In this study the following investigations are being made: (a) Determination is being made of the amount of fluorine ingested from the daily diet, including baby food preparations. (b) Dental tissue is being examined for fluorine content. Dental cements have been analyzed and teeth containing silicate cements have been eliminated. (c) At autopsies a section of the crest of the ilium is removed and analyzed for fluorine content. The tooth buds, mandible, and maxilla, and the femurs of fetuses are being analyzed to determine the fluorine content of the tissues at birth. (d) A careful check is being kept on the Evanston water supply. Daily samples are being collected at various distribution points throughout the city and at the filtration plant to be certain there is one part per million of fluorine in the drinking water.

The study began in December, 1945, and is being conducted jointly by the Evanston Health Department, the Walter G. Zoller Memorial Dental Clinic of the University of Chicago, and the Department of Public Health Dentistry of the Illinois State Health Department. Dr. J. R. Blayney, Director of the Zoller Dental Clinic, is in general supervision of the study. The purpose of this study is to determine whether or not the addition of one part per million of fluorine to the public water supply would reduce the incidence or retard the rate of progress of dental caries. This study is to be continued until at least one group of children that has been exposed to fluorine throughout life—prenatal and postnatal—has reached the age of 14 years.

METHODS OF ANALYSIS AND MATERIALS

The determinations of all the materials, except the water samples, were made by the method of Willard and Winter,[1] using the modifications of the Association of Official Agricultural Chemists.[2] The daily water analyses are done using the Scott modification (Goudey). Magnesium acetate is employed as the fluorine fixative on all organic materials. Potassium fluosilicate is used as the primary fluorine standard. This standard was prepared and its purity tested in the laboratories of the United States Food and Drug Administration, Washington, D. C. In the water analysis sodium fluoride is used as the standard.

The stills are of Pyrex glass with all glass connections as recommended by the Associate Referee of the Food and Drug Administration, Washington, D. C.

Read at the Twenty-Fifth General Meeting of the International Association for Dental Research, Chicago, June 21-22, 1947 (*J. D. Res.* 26: 1947).

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The flasks are a Claissen type distilling flask of 125 ml. size. All precautions were taken to maintain a minimum distillation blank, including the use of platinum dishes when ashing the material.

In obtaining materials to be analyzed for fluorine content—foods, bones, and teeth—care is taken to use only those from Evanston and vicinity. All the foods are purchased upon the open market in Evanston; the teeth are supplied by the local dentists; the bones (crest of ilium) are obtained from necropsies; the fetuses and stillbirths are from hospitals in the metropolitan area. The chemicals used are all of chemically pure quality.

EXPERIMENTAL

A. Occurrence of Fluorides in Foods

It is known that fluorine compounds are present in numerous foods, the amount varying with the type and the source of the foods. A summary of the fluorine content of foods has been prepared by McClure,[3] and Clifford.[4] The foods analyzed in the Evanston laboratory included soups, rice, baby foods, cereals. The results have been thought worthy of publication because they represent recent analysis by the accepted method. A summary of the soups will be found in Table I, of the other foods, in Table II. The soups are nationally advertised brands.

TABLE I
FLUORINE CONTENT OF SOUP

SOUP*	PPM FLUORINE
Beef Noodle "A"	1.00
Ox Tail "A"	0.85
Chicken Noodle "A"	2.80
Scotch Broth "A"	0.40
Chicken Gumbo "A"	1.00
Pepper Pot "A"	0.80
Vegetable "B"	1.10
Chicken Noodle "B"	1.60
Beef Noodle "B"	0.98
Gumbo Creole "B"	0.65
Pepper Pot "B"	0.80

*"A" and "B" are trade names of the soups

TABLE II
FLUORINE CONTENT OF FOODS

FOODS ANALYZED	PPM FLUORINE
Rice, polished	1.0 to 2.6
Flour, white	0.0
Oatmeal	1.4
Sucrose	1.8
Spry	2.1
Pablum	11.2
Pabina	5.3
Postum	0.8
Hydrolyzed vegetable protein	1.8
Sodium glutamate	8.4

The analyses showed the chicken soups to be higher in fluorine content than the other soups analyzed. In an attempt to explain this fact some of the in-

gredients were analyzed. The results obtained reveal: chicken bones, 205 ppm fluorine, and vegetable protein, 1.8 ppm fluorine. The other soups contained sodium glutamate which has a fluorine content of 8.4 ppm fluorine. This may be an explanation of the fluorine content of the soups and of the higher fluorine content of chicken soup. In an analysis of the baby foods, the Pablum was found to contain 11.2 ppm fluorine, and Pabina, 5.3 ppm fluorine. The fluorine content of these foods may be due to the bone meal they contain, which has a naturally high fluorine content.

B. Occurrence of Fluorine in Teeth

In the analysis of teeth some difficulties were encountered at first. It was found that teeth with silicate cement fillings had to be eliminated. An analysis of six cement powders is reported in Table III. Paffenbarger, Schoonover, and Souder[5] have shown that dental silicate cements range from 11 per cent to 15 per cent in fluorine content. The fluorine is used in the flux as an aid to fuse the cements.

TABLE III

FLUORINE CONTENT OF DENTAL SILICATE CEMENTS

CEMENT ANALYZED	PER CENT FLUORINE
Cement and liquid, golden brown	0.000038
Red copper cement and liquid	0.000070
Black copper cement, powder and liquid	0.000036
Porcelain and liquid	12.100000
Porcelain and liquid	13.800000
Zinc oxide, rosin with eugenol, olive oil, liquid	0.00025

The results recorded in this study are only on teeth without fillings. This limiting factor has reduced the number of teeth available for analysis. Table IV is a summary of whole teeth analyzed in the past year.

Armstrong,[6] and Armstrong and Brekhus[7] have shown the mean fluorine content of enamel of sound teeth to be 0.0111 per cent, dentin of sound teeth 0.0169 per cent, and in some cases the enamel of sound teeth to be as high as 0.0204 per cent. They also showed that carious teeth were lower in fluorine content—carious enamel 0.0069 per cent to 0.0184 per cent. The results of the Evanston Study showed the enamel and dentin of sound teeth to vary from 0.0104 per cent to 0.0287 per cent fluorine with an average of 0.0202 per cent fluorine. The fluorine content of the enamel and dentin of carious teeth was 0.0045 per cent to 0.0270 per cent with an average of 0.0133 per cent. All of these teeth were analyzed before fluorine was added to the potable water supply.

C. Occurrence of Fluorine in Bone and Fetus

A metropolitan hospital has furnished sections of the crest of the ilium taken at autopsies. All of the bones were made fat free and dry, before analysis. The age, sex, and length of residence in Evanston of the patients were not furnished in some cases. An average fluorine content of 0.0376 per cent was obtained from the thirteen specimens examined, which is higher than the average fluorine content of the teeth analyzed. Table V shows the results of the

TABLE IV

FLUORINE CONTENT OF WHOLE TEETH

AGE	SEX	PER CENT FLUORINE	TOOTH NO.	DEGREE OF CARIES
11	M	0.0104	V | | V | III	None
12	M	0.0194	V |	Moderate
12	M	0.0137	| 4	Moderate
18	F	0.0116	| 1	None
18	F	0.0121	1 |	None
18	F	0.0137	2 |	None
18	M	0.0045	| 8	Excessive
19	F	0.0055	| 8	Moderate
19	F	0.0080	8 |	Moderate
20	F	0.0270	7 |	Excessive
22	F	0.0112	| 3	Moderate
33	M	0.0061	| 3	Excessive
37	M	0.0271	2 |	None
37	F	0.0112	| 8	Excessive
51	F	0.0215	7 |	None
51	F	0.0255	8 |	None
51	F	0.0176	6 |	None
51	F	0.0227	| 5	None
51	F	0.0287	| 7	None
51	F	0.0179	7 |	None
51	F	0.0216	| 4	None
51	F	0.0212	| 5	None
51	F	0.0234	| 6	None
51	F	0.0212	| 7	None
60	M	0.0267	3 |	Moderate
60	M	0.0268	| 3	None

analyses. Boissevain and Drea[8] showed the normal fluorine content of bone to be 0.0700 per cent. This is higher than has been found in the Evanston area.

The crest of the ilium was selected because it can be obtained without mutilating the body, as well as allowing use of the same bone, and it is the point at which the psoas ligament is attached. It is believed that the point at which a ligament is in contact with a bone is the region of greatest concentration of fluorine.

In investigation of the fluorine content of the bones of mature individuals, the question arose as to the amount of fluorine transferred from the mother to the fetus through the placental circulatory system. It has been shown in rats that the fluorine content of the fetus, removed by cesarean section before suckling, and of the newborn (from birth to four days old) varies from 1.99 to 0.28 ppm.[9]

TABLE V

FLUORINE CONTENT OF BONE

AGE	SEX	PER CENT FLUORINE	LENGTH OF RESIDENCE IN EVANSTON
32	M	0.0323	3 years
46	F	0.0371	19 years
51	F	0.0207	51 years
58	F	0.0164	50 years
61	M	0.0365	11 years
62	M	0.0428	32 years
65	M	0.0294	35 years
70	M	0.0396	37 years
84	M	0.0505	10 years
*		0.0288	
*		0.0324	
*		0.0473	
*		0.0753	

*Age, sex, and length of residence unavailable.

TABLE VI

FLUORINE CONTENT OF FETUS BONES AND TEETH

FETUS NO.	TISSUE ANALYZED	PPM F	L.M.P.*	DATE OF DELIVERY	LENGTH IN CM.	WEIGHT IN GRAMS
1	Femur	29.0	2/28/46	11/30/46	55	4,790
	Mandible and Maxilla	27.0				
	Tooth Buds	17.0				
2	Femur	20.0	2/14/46	11/20/46	43	1,950
	Mandible and Maxilla	19.0				
	Tooth Buds	13.0				
3	Femur	16.0	8/ 8/47	5/16/47	38	1,125
	Mandible and Maxilla	15.0				
	Tooth Buds	8.7				
4	Femur	27.0	8/13/46	5/17/47	40	1,435
	Mandible and Maxilla	24.0				
	Tooth Buds	13.4				
5†	Femur	24.0			32	1,300
	Mandible and Maxilla	24.0				
	Tooth Buds	15.0				
6	Femur	23.5	6/19/46	4/ 6/47	52	3,180
	Mandible and Maxilla	20.0				
	Tooth Buds	15.0				
7	Femur	14.0	6/23/46	4/ 2/47	56	4,660
	Mandible and Maxilla	15.0				
	Tooth Buds	9.0				
8	Femur	10.6	8/15/46	3/14/47	37	1,000
	Mandible and Maxilla	9.3				
	Tooth Buds	8.0				

*Last menstrual period.
†Anencephalic monster.

In cooperation with this study a metropolitan hospital has supplied at various times, fetuses of approximately nine months gestation. See Table VI for a detailed report on this subject. The left and right femurs, mandible and maxilla, and the tooth buds were used for the analyses. All of the bones and tooth buds were made fat-free and dry. A summary of the analyses shows the

average fluorine contents to be: femur—20 ppm F, mandible and maxilla—19 ppm F, and the tooth buds—12.5 ppm F.

D. Fluorine in the Water Supply

Since February 11, 1947, when fluorine was added to the Evanston water supply in the amount of one part per million, daily fluorine determinations have been made on water samples taken in the filtration plant and the distribution system. During the first week that fluorine was added to the public water supply a daily sample was taken at nine points in the distribution system to determine the rate at which the fluorine was being distributed throughout the city. Table VII shows the results found at that time.

TABLE VII

FLUORINE CONCENTRATION IN THE WATER DURING THE FIRST WEEK IT WAS ADDED*

DISTRIBUTION POINT	PARTS PER MILLION FLUORINE					
	2/11/47	2/12/47	2/13/47	2/14/47	2/15/47	2/17/47
Filtration lab.	0.05	0.95	1.00	0.95	1.00	1.00
No. 1	0.00	0.35	0.85	0.90	1.00	1.00
No. 3	0.00	0.43	0.80	0.90	1.00	1.00
No. 6	0.00	0.45	0.90	0.95	1.00	1.00
No. 8	0.00	0.80	0.95	1.00	1.00	1.00
No. 13	0.00	0.80	0.95	1.00	1.00	1.00
No. 14	0.00	0.15	0.75	0.85	0.90	0.95
No. 15	0.00	0.90	0.96	1.00	1.00	1.00
No. 4A	0.00	No test	0.50	0.80	0.90	0.95

*All samples collected between 9:00 A.M. and 10:00 A.M.

To date no difficulties have been encountered in maintaining a constant level of 1.00 ppm fluorine in the potable water supply. It has been found that the fluorine content of the water at the distribution points is approximately 2 per cent lower than the fluorine content of the water at the filtration plant, the point of application. The farthest distribution point at which a sample is taken is about five miles from the filtration plant. Fluorine is added to the water supply in the form of sodium fluoride, a 95 per cent pure dense, white powder. It is fed into the system by means of a gravity feed machine.

SUMMARY

The question of the advisability of adding fluorine to public drinking water supplies as a method to help control dental caries is now a major public health problem. One phase of this work is in progress in the chemical laboratory of Evanston. Chemical analyses of the foods available in the Evanston markets are made to determine the fluorine content. Permanent teeth which must be extracted and deciduous teeth which are shed are used to determine the fluorine content of dental tissues. The crest of the ilium and fetuses are used to determine the fluorine content of bones. Daily chemical analyses are made to determine the fluorine content of water samples taken from all parts of the city. It is believed that these analyses will furnish considerable information of value on the control of dental caries by the use of public health methods.

The physiologic action of the fluoride ion needs further investigation, even though it has been studied at some length. Further research should be encouraged on the methods of analysis. There is need for more complete data regarding the occurrence of fluorides in foods, bones, and teeth.

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REFERENCES

1. Willard, H. H., and Winter, O. B.: Volumetric Method for Determination of Fluorine, *Indust. Engin. Chem. (Anal. Ed.)* **5**: 7-10, 1933.
2. Fluorine, Association of Official Agricultural Chemists, *J. Assoc. Official Agr. Chem.* **27**: 90, 1944.
3. McClure, F. J.: Fluorides in Food and Drinking Water, *Natl. Inst. Health Bull.* **172**: 1-53, 1939c.
4. Clifford, P. A.: Report on Fluorine, *J. Assoc. Official Agr. Chem.* **28**: 1945.
5. Paffenbarger, G. C., Schoonover, I. C., and Souder, W.: Dental Silicate Cements, *J. A. D. A.* **25**: 32-87, 1938.
6. Armstrong, W. D.: Fluorine Content of Enamel and Dentin of Sound and Carious Teeth, *J. Biol. Chem.* **119**: V-VI, 1937.
7. Armstrong, W. D., and Brekhus, P. J.: Fluorine Content of Enamel of Sound and Carious Teeth, *J. D. Res.* **16**: 309-310, 1939.
8. Boissevain, C. H., and Drea, W. F.: Spectroscopic Determination of Fluorine in Bones, Teeth, and Other Organs in Relation to Fluorine in Drinking Water, *J. D. Res.* **13**: 495-500, 1933.
9. Zoller Dental Clinic, Germ Free Animal Study (Unpublished data).

OBSERVATIONS ON INDUCED CARIES IN RATS

VI. Summary Results of Various Modifications of Food and Drinking Water

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THE cariouslike dental lesions induced in the white rat by diets containing large corn or rice particles are inhibited to a marked degree by fluoride,[1-5] iodoacetate,[1, 5] and penicillin.[6-8] These experimental results with fluoride are in agreement with the epidemiological observations of Dean and his co-workers[9] who ascertained a remarkable correlation between fluoride in communal water supplies, and a reduced prevalence of human dental caries. With regard to the effect of penicillin, Kniesner and Hill[10] observed a marked inhibition of oral lactobacillus acidophilus in young adults using a penicillin dentifrice. The oral lactobacillus acidophilus counts in rats are also reduced by penicillin, together with a notable reduction in caries.[6]

Much criticism has been directed against the production of lesions in rats' molar teeth by means of a coarse-particle diet which introduces the complicating factor of fractures, and an analogy between experimental rat caries produced by such diets and human dental caries has been questioned, with good reason. It is a primary aim of experimental caries research to produce dental caries in the white rat without the coarse-particle diet complication. Although some success has been achieved in this regard,[11-13] variable factors related to diet, prenatal treatment, and the animal's inherent susceptibility are not yet resolved.

The induced form of rat caries, in spite of its uncertain etiology, may serve a useful purpose in classifying other agents besides fluorine, for example, which are suggested as possible dental caries inhibitors or of substances suggested as caries accelerators. In addition to the prospect of finding agents suitable for human caries control, the results may be instrumental in identifying some of the basic reactions of caries etiology. It may be recalled that Miller,[1] who first reported the inhibitory effects of fluoride and iodoacetate on induced rat caries, based his interest in these substances on their notable anti-enzymatic properties. The inhibitory effects of these two substances are suggestive of a role of glycolysis through the phosphorylation mechanism in caries etiology. Specific inhibitors of glycolysis or of other enzyme systems having been identified with experimental caries, may be found later to seek a similar target in the human organism.

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Other results with experimental rats, showing an effect of citrate,[14] oxalate,[15] certain acid drinks and beverages,[16] and raw tomato[12] on rats' oral tooth surfaces, suggest that some constituents of ordinary foods and beverages may be a hazard to human dental health, particularly through oral tooth surface effects. Experimental dental studies, therefore, have proved fruitful in these studies in the important and largely unexplored field of the direct chemical effects of diet factors on oral tooth surfaces.

EXPERIMENTAL

A routine regimen was followed in all the experiments reported in Table I. Groups of rats aged 23 to 25 days, sixteen to twenty-three liter mates in control and corresponding test groups, were on a test period of about fifteen weeks. The corn-meal (Hoppert-Webber-Canniff) diet[17] was fed ad libitum. Water intake was not controlled, the results being related, therefore, to the concentration of a particular factor in the food or drinking water. The molar teeth were examined under low-power (10X) magnification, and scored according to the system of Cox, Dodds, Dixon, and Matuschak.[18] The results pertaining to fluoride alone (in food and drinking water), iodoacetate, and penicillin, have been published elsewhere.[4-6] It should be noted particularly that comparisons are valid only as between a test group and its corresponding control group. It is apparent in the data for the various control groups that it was not possible to produce consistently a high incidence of caries with the corn-meal diet. In one control group (Series 14) it may be noted that only 25 per cent of the rats were diagnosed as carious, whereas in other control groups (Series 6 and 10) all of the rats were carious. The cause of this deviation is not known but may be attributed, perhaps, to variation in rats' inherent susceptibility[11] and to different sources of the diet materials, particularly the corn which might influence the physical properties of the ration. A commercial brand of yellow hominy grits was fed, except during the war years, when shelled corn was ground in the laboratory to the desired particle size. This later source of dietary corn was less satisfactory for the production of caries than the commercial yellow grits.

DISCUSSION

Fluoride.—A problem of practical interest in fluorine-dental caries epidemiology is the possibility of associative effects of other water-borne elements, on the fluoride-caries inhibition. For example, Dean[19] has called attention particularly to the variability in total hardness of fluoride waters, and its apparent lack of any correlation with the caries-inhibitory effects of fluoride waters. Other variable water constituents might be cited, as for example 352 ppm sulfate in Galesburg, Ill., water, versus 98 ppm sulfate in Elmhurst, Ill., water, both waters containing similar fluorine, i. e., about 1.8 ppm, and having similar anticaries effects.[9]

It was of some interest to study this problem experimentally, therefore, by adding sodium sulfate and magnesium chloride to a fluoride water. These results are reported in Series 5 and 6 (Table I) with no evidence that sodium

TABLE I

EFFECTS OF VARIOUS SUBSTANCES ADDED TO FOOD AND DRINKING WATER ON INDUCED RAT CARIES

EXP. NO.	MODIFICATION OF FOOD AND DRINKING WATER	ANIMALS (NO.)	LITTERS (NO.)	RESULTS OF CARIES DIAGNOSIS: RATS WITH CARIES (%)	CARIOUS TEETH (%)	CARIOUS AREAS (%)	AV. CARIES SCORE (NO.)	SCORE PER CARIOUS RAT (NO.)
	Fluoride and Iodoacetate							
1	Control	26	15	80.0	24.4	20.6	18.0	22.3
1a	5 ppm F as NaF in water	25	18	88.0	24.3	20.0	17.9	20.3
1b	50 ppm F as NaF in water	25	18	52.0	10.7	5.5	4.0	7.6
2	Control	27	5	100.0	35.2	31.4	27.6	27.6
2a	10 ppm F as NaF in water	24	8	79.2	26.7	18.8	15.6	19.7
2b	100 ppm F as NaF in water	26	10	57.7	13.8	7.8	6.2	16.1
3	Control	27	5	92.6	36.1	32.2	28.7	31.0
3a	125 ppm F as NaF in water	26	6	30.8	3.5	1.4	0.7	2.1
4	Control	25	21	76.0	24.7	15.8	10.6	13.9
4a	125 ppm F as NaF in food	25	20	12.0	1.0	0.4	0.2	1.3
4b	200 ppm $CH_2 \cdot I \cdot COOH$ in food, 20 ppm in water	24	12	33.3	6.6	2.7	1.5	4.5
	Fluoride, Magnesium Chloride, and Sodium Sulfate. Fluoride in Combination With Magnesium Chloride and Sodium Sulfate							
5	Control	23	21	95.7	27.5	20.6	17.1	17.9
5a	10 ppm F as NaF in water	23	21	69.6	14.9	10.5	8.2	11.8
5b	2000 ppm SO_4 as Na_2SO_4 in water	23	21	95.7	27.9	19.3	16.3	17.0
5c	10 ppm F as NaF, 2000 ppm SO_4 as Na_2SO_4 in water	23	20	87.0	20.7	12.8	10.7	12.3
6	Control	23	21	100.0	27.9	26.3	15.7	15.7
6a	10 ppm F as NaF in water	20	19	80.0	22.1	16.6	14.0	17.4
6b	10 ppm F as NaF, 2000 ppm SO_4 as Na_2SO_4 in water	24	21	95.8	23.6	15.8	13.1	13.7
6c	500 ppm Mg as $MgCl_2$ in water	22	21	95.5	28.0	24.2	20.4	21.3
6d	10 ppm F as NaF, 500 ppm Mg as $MgCl_2$ in water	22	20	86.4	23.9	19.4	14.5	16.8
	Sodium Iodide, Free Iodine, and Sodium Fluoride Plus Free Iodine							
5	Control	23	21	95.7	27.5	20.6	17.1	17.9
5d	15 ppm iodine (free) in water	23	22	91.3	29.0	22.8	19.3	21.1
5e	15 ppm iodine (free), 10 ppm F as NaF in water	23	20	78.3	25.0	18.7	13.8	17.6
5f	150 ppm iodine as sodium iodide in water	22	14	86.4	26.1	22.5	19.1	22.1
	Sodium Hydroxide, Calcium Chloride, Thioglycolic Acid, Silver Nitrate and Potassium Thiocyanate							
7	Control	21	18	85.7	26.6	24.1	20.7	24.2
7a	N/200 NaOH, pH 11.5	22	19	95.5	34.1	31.6	28.1	29.4
7b	1500 ppm Ca as $CaCl_2$ in water	22	20	86.4	28.0	23.4	17.1	19.8
7c	100 ppm $SH \cdot CH_2 \cdot COOH$ in water	18	9	100.0	43.1	36.3	31.5	31.5
7d	100 ppm $AgNO_3$ in water	18	19	55.5	8.3	16.8	15.8	28.4
8	100 ppm KCNS in water	21	15	95.2	25.4	22.1	18.7	19.7
	Iron, Copper, and Zinc							
9	Control	12	10	91.7	34.0	23.8	18.3	20.4
9a	250 ppm Fe as $FeCl_3$ in water	12	10	58.3	17.4	8.6	5.4	9.3
9b	250 ppm Cu as $CuSO_4$ in water	12	10	50.0	13.9	7.1	5.3	10.5
10	Control	24	24	100.0	33.0	19.0	13.9	13.9
10a	500 ppm Fe as Ferric Citrate in water	24	24	87.5	31.9	23.4	16.8	19.2
10b	250 ppm Fe as Ferric Citrate in water and food	24	24	91.7	27.4	15.9	11.2	12.2
10c	500 ppm Cu as $CuSO_4$ in food	24	24	87.5	31.9	23.4	16.8	19.2
10d	250 ppm Cu as $CuSo_4$ in water and in food	24	22	90.9	27.7	14.0	8.3	9.2
10e	250 ppm Zn as $ZnSO_4$ in water and in food	24	23	100.0	33.7	21.6	16.0	16.0

TABLE I—CONT'D

EXP. NO.	MODIFICATION OF FOOD AND DRINKING WATER	ANIMALS (NO.)	LITTERS (NO.)	RESULTS OF CARIES DIAGNOSIS: RATS WITH CARIES (%)	CARIOUS TEETH (%)	CARIOUS AREAS (%)	AV. CARIES SCORE (NO.)	SCORE PER CARIOUS RAT (NO.)
	Urea							
7	Control	21	18	85.7	26.6	24.1	20.7	24.2
7e	Urea, 1000 ppm in water, 1% in food	21	19	76.2	14.3	9.7	7.9	10.3
11	Control	36	13	69.4	14.4	6.7	5.3	7.6
11a	Urea, 500 to 1000 ppm in water, 2% in food	39	10	54.0	7.9	3.2	2.2	4.1
	Penicillin							
12	Control	82	43	50.0	6.8	0.0	2.7	5.4
12a	75 units of penicillin in food and water	82	43	0.0	0.0	0.0	0.0	0.0
	Sodium Nitrate and Ammonium Nitrate							
13	Control	36	14	61.1	11.1	2.7	4.2	7.0
13a	1500 ppm NO_3 as $NaNO_3$ in water 0.5% NO_3 in food	36	14	16.6	2.3	0.5	0.7	4.1
14	Control	24	10	25.0	6.6	2.3	1.1	4.3
14a	1500 ppm NO_3 as NH_4NO_3 in water 0.5% NO_3 in food	31	10	3.2	0.4	0.1	0.1	4.0
15	Control	28	11	96.4	32.1	16.3	14.1	14.7
15a	1500 ppm NO_3 as $NaNO_3$ in water 0.5% NO_3 in food	28	11	78.6	22.9	9.0	6.9	8.8
16	Control	19	6	84.3	16.2	6.5	4.6	5.4
16a	500 ppm NO_3 as $NaNO_3$ in water	21	6	66.6	11.5	5.6	4.7	7.0

sulfate or magnesium chloride in the quantities fed influenced the anticaries response of 10 ppm fluorine. With and without the presence of sodium sulfate and magnesium chloride there is apparent a slight inhibitory effect of 10 ppm fluorine in these two series of studies.

There are several reports of an association of relatively small quantities of fluorine with iodine metabolism via an involvement of the thyroid gland.[20-22] Although the evidence with regard to these reports is not too convincing, the possibility of a physiological relationship between fluorine and iodine is suggested. In Series 5 (Table I) free iodine (15 ppm) was added to rats' drinking water which also contained 10 ppm fluorine. Apparently this quantity of iodine had no influence on the usual inhibitory effect of 10 ppm fluorine.

Sodium Sulfate, Magnesium Chloride, and Calcium Chloride.—These important minerals were tested in Series 5, 6, and 7 for their influence on rats' caries experience, but only negative results are apparent in the data (Table I). It would not be predicted from these results that these substances in communal water supplies are a factor in the community caries index. Magnesium may be of special interest because of its unusual activity as an enzyme activator.[23] In Series 7 (Table I) a strongly alkaline drinking fluid, i. e., N/200 NaOH, pH 11.5, also had no apparent influence on rats' caries response, whereas current acid decalcification theories suggest that the severity of caries would be reduced by the presence of so much alkali in the rat's oral cavity.

Iodide, Thioglycolic Acid, Silver Nitrate, and Potassium Thiocyanate.—This group of substances was tested for various reasons, none of these chemicals

having a particular identity with caries etiology. Potassium thiocyanate is a peculiar and as yet an unqualified constituent of saliva, whose connection with caries etiology has been intimated. Its effects on rat caries apparently are negative (Series 8, Table I). Silver nitrate has had some study (in vitro) as a possible prophylaxis against human dental caries.[24] Among the limited number of rats in Series 7d, there seemed to be a somewhat significant reduction of caries due to 100 ppm silver nitrate in the drinking water.

Iodide has been reported to increase amylase activity.[23] Its involvement in rat caries at a level of 150 ppm iodine as potassium iodide in rats' drinking water was not apparent (Series 5, Table I). Similarly, free iodine amounting to 15 ppm in the drinking water of one group of twenty-three rats as compared with twenty-three controls had no effect on the rats' caries experience.

Iron, Copper, and Zinc.—An unusual number of enzyme systems are affected by these particular elements.[23] As inhibitors or activators of dental caries they have a connection suggested through their association with enzyme systems. There was some indication in a preliminary trial (Series 9) that iron and copper were caries-inhibitory. This result was not repeated, however, in the experiments of Series 10, although there may be some slight caries reduction in the copper groups, particularly in the caries scores of Series 10d, (250 ppm Cu as $CuSO_4$ in food and water). There is definitely no indication that Zn has any caries-inhibitory properties. The result with thioglycolic acid suggests an agent capable of inducing caries. All of the rats fed this substance had caries, present in a more rampant form than occurred in any other series of the studies.

Urea.—Urea has had some study as a possible caries inhibitor particularly by Stephan.[25] "Evidence is presented which indicates that the effect of carbohydrates in lowering the pH of plaques can be largely overcome for a period up to twenty-four hours by the application of urea in 40 to 50 per cent concentration for a time of about four minutes."[25] Theoretically urease activity in plaque material is greatly accelerated by massive doses of urea, giving rise to a strong local acid-neutralizing effect. In Experiments 7 and 11, we studied the effects of 500 to 1000 ppm urea in water and 1 and 2 per cent urea in the diet. In both these urea-groups, caries was reduced below the control groups to an extent which appears to be significant. The reduction occurs not only in the percentage of rats having caries but also in the number of surfaces involved and in the average caries scores. Urea, it would appear, had some inhibitory effects in which its base-producing properties may or may not be involved. Urea also apparently has very limited association with enzyme systems.[23] A pronounced effect of urea on caries, presumably, requires somewhat massive doses.

Penicillin.—The effect of penicillin, including the reduction in lactobacillus acidophilus counts, has been discussed in a previous publication.[6] This result has been repeated by Kniesner and Hill[10] and by Zander and Bibby,[7] and the action of this antibiotic substance attributed to a change in the rats' oral flora.

The results with penicillin seem to furnish "additional evidence of an association of *L. acidophilus* and perhaps other bacteria with dental caries etiology."[6]

Nitrate.—In the numerous water analyses which have been made in this laboratory by Elvove, in connection with epidemiological studies of fluorosis and dental caries,[9, 26] nitrate determinations were always included. While most of these nitrate figures are of the order of 1.0 to 3.0-4.0 ppm, comparatively much higher figures for nitrate have been found to occur in some drinking waters.

These water analyses suggested, therefore, as a matter of particular interest[27] an experimental study of the effect of high nitrate concentrations in food and drinking water on dental caries as induced in the experimental rat. Accordingly the experiments reported in Table I, Series 13 through 16a, were conducted to make a preliminary test of this suggestion. In all cases the groups receiving nitrate in food or water had less caries than their corresponding controls. The effects of 1500 ppm nitrate are quite pronounced although 500 ppm also seems to have been instrumental in reducing slightly the rats' caries experience. The consistent trend of these results agrees with the suggestion of a possible role of water-borne nitrate in dental caries etiology.[27]

In addition to the rat studies described, it is of interest to note that Holmes, Parkinson, Wertz, and McKay[12] report that feeding sulfasuxidine, dl– valine, pulsatilla, and dl– tryptophane "did not alter caries in rats."*

In addition to the various substances reported in all these rat experiments there are, obviously, many other enzyme activators and inhibitors, chemotherapeutic and antibiotic agents, available for similar study. Likewise, there are numerous chemicals in ordinary foods and drinking waters which should be studied for their possible involvement in caries etiology. Aside from bacterial activity engendered by food residues remaining on the tooth, the possibilities of specific food and water-borne chemicals acting directly on oral tooth surfaces are worthy of special consideration.

SUMMARY

The inhibitory effects of fluorine on induced rat caries are apparent at a level of 10 ppm in drinking water and are independent of any association with sodium sulfate, magnesium chloride, or free iodine in the water. Urea inhibited rat caries to a limited extent, when given in somewhat excessive quantities in the food and water. Nitrate added to the drinking water and food seemed consistently to inhibit experimental rat caries. Penicillin and iodoacetate have been reported from previous studies as markedly caries-inhibitory. The following substances gave no indication of any pronounced effect on induced rat caries: iron, copper, zinc, calcium chloride, sodium sulfate, magnesium chloride, potassium iodide, free iodine, potassium thiocyanate, and a strongly alkaline drinking fluid.

REFERENCES

1. Miller, B. F.: Inhibition of Experimental Dental Caries in the Rat by Fluoride and Iodoacetic acid, *Proc. Soc. Exper. Biol. & Med.* **39**: 389, 1938.
2. Finn, S. B., and Hodge, H. C.: The Inhibition of Experimental Dental Caries by Fluorine, *J. D. Res.* **18**: 252, 1939.

*More recently Schlack, Howell, Taylor, Berzinskas, and Aborn[28] found that oxalates in diet and drinking water had no effect on caries in rats fed a coarse corn particle diet.

3. Cox, G. J., Matuschak, M., Dixon, S. F., Dodds, M. L., and Walker, W. E.: Experimental Dental Caries. IV. Fluorine and Its Relation to Dental Caries, *J. D. Res.* **18**: 481, 1939.
4. McClure, F. J.: Observations on Induced Caries in Rats. III. Effect of Fluoride on Rat Caries and on Composition of Rats' Teeth, *J. Nutrition* **22**: 391, 1941.
5. McClure, F. J., and Arnold, F. A., Jr.: Observations on Induced Dental Caries in Rats. I. Reduction by Fluoride and Iodoacetic Acid, *J. D. Res.* **20**: 97, 1941.
6. McClure, F. J., and Hewitt, W. L.: The Relation of Penicillin to Induced Rat Dental Caries and Oral L. Acidophilus, *J. D. Res.* **25**: 441, 1947.
7. Zander, H. A., and Bibby, B. G.: Laboratory and Animal Studies on Effect of Penicillin on Caries Activity, *J. D. Res.* **26**: 365, 1947.
8. Webman, H., Kniesner, A. H., and Hill, T. J.: Effect of Penicillin on Incidence of Dental Caries in Rats Fed a Coarse Diet, *J. D. Res.* **26**: 455, 1947.
9. Dean, H. T.: Epidemiological Studies in the United States in, Dental Caries and Fluorine ed. by F. R. Moulton, Am. Assoc. Adv. Science, Washington, 1946.
10. Hill, T. J., and Kniesner, A. H.: Laboratory and Animal Studies on the Effect of Penicillin on Caries Activity, *J. D. Res.* **26**: 454, 1947.
11. McClure, F. J.: Observations on Induced Caries in Rats. V. Effect of Excessive Sugar in the Diet, *J. D. Res.* **24**: 239, 1945.
12. Holmes, J. O., Parkinson, L. R., Wertz, A. W., and McKay, B. V.: Dental Caries in the Rat. Proceedings Federated Biological Societies, 6, 1, 409, 1947.
13. Sognnaes, R. F.: A Possible Role of Food Purification in the Etiology of Dental Caries, *Science* **106**: 448, 1947.
14. McClure, F. J., and Ruzicka, S. J.: The Destructive Effect of Citrate vs. Lactate Ions on Rat's Molar Tooth Surfaces, In Vivo, *J. D. Res.* **25**: 1, 1946.
15. Gortner, R. A., Jr., McCay, C. M., Restarshi, J. S., and Schlack, C. A.: Some Effects of Dietary Oxalate on the Teeth of White Rats, *J. Nutrition* **33**: 121, 1946.
16. McClure, F. J.: The Destructive Action In Vivo, of Dilute Acids and Acid Drinks and Beverages on the Rat's Molar Teeth, *J. Nutrition* **26**: 251, 1943.
17. Hoppert, C. A., Webber, P. A., and Canniff, T. L.: The Production of Dental Caries in Rats Fed an Adequate Diet, *Science* **74**: 77, 1931.
18. Cox, G. J., Dodds, M. L., Dixon, S. F., and Matuschak, M. C.: Experimental Dental Caries. III. A System of Recording Corn Meal Caries in Rats, *J. D. Res.* **18**: 469, 1939.
19. Dean, H. T.: Domestic Water and Dental Caries, *J. Am. Water Works Assn.* **35**: 1161, 1943.
20. Seevers, M. H., and Braun, H. A.: Effect of Sodium Fluoride Upon Experimental Thyroid Poisoning, *Proc. Soc. Exper. Biol. & Med.* **33**: 228, 1935.
21. Phillips, P. H., English, H., and Hart, E. B.: The Augmentation of the Toxicity of Fluorosis in the Chick by Feeding Desiccated Thyroid, *J. Nutrition* **10**: 399, 1935.
22. Kraft, K.: The Biochemistry of Fluorine. I. Antagonism of Fluorine and Thyroxine, *Ztschr. f. physiol. Chem.* **58**: 245, 1936.
23. Sumner, J. B., and Somers, G. F.: Chemistry and Methods of Enzymes, New York, 1943, Academic Press, Inc.
24. Hill, T. J., and Arnold, F. A., Jr.: The Effect of Silver Nitrate in the Prevention of Dental Caries, *J. D. Res.* **16**: 23, 1937.
25. Stephan, R. M.: The Effect of Urea in Counteracting the Influence of Carbohydrates on the pH of Dental Plaque, *J. D. Res.* **22**: 63, 1943.
26. Dean, H. T., Arnold, F. A., Jr., and Elvove, E.: Domestic Waters and Dental Caries. V. Additional Studies of the Relation of Fluoride Domestic Waters to Dental Caries Experience in 4,425 White Children Age 12-14 Years of Thirteen Cities in Four States, *Pub. Health Rep.* **57**: 1115, 1942.
27. Elvove, E., and Dean, H. T.: Private communication.
28. Schlack, C. A., Howell, S. R., Taylor, B. L., Berzinskas, V. J., and Aborn, M. H.: The Role of Oxalates in Rat Dental Caries, *J. D. Res.* **26**: 383, 1947.

A METHOD FOR QUANTITATIVE DETERMINATIONS OF MICROORGANISMS IN CARIOUS AND NON-CARIOUS TEETH OF THE COTTON RAT

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FOR many years attention has been focused on the role of oral bacteria in the etiology of dental caries. The majority of the work on the bacteriological aspects of caries has been done on human teeth and saliva, while relatively little work has been done on the bacteria involved in dental caries produced in experimental animals. Attempts have been made to increase caries production in experimental animals by incorporation of human lactobacillus strains in the diet of the animals.[1] Qualitative studies of the buccal flora have been based on the identification of organisms obtained from culturing carious lesions[2] and oral swabbings.[3] Harrison[4] has reported quantitative studies of organisms found in carious teeth of the white rat. In recent studies with the Syrian hamster, specimens for study were obtained by aspirating aseptically the material from molar tooth surfaces.[5]

The effect of diet on the incidence and extent of dental caries in the cotton rat has been the subject of a series of investigations.[6-8] In the light of other investigations, it seemed important to study the bacteriological aspects of this problem since no work of this kind has been done with the cotton rat. Of primary importance was the development of a satisfactory technic for making quantitative bacteriological counts of the whole tooth. Since carious lesions in the molar teeth of the cotton rat occur deep within the occlusal fissures, surface methods were deemed inadequate.

METHODS

To obtain numbers of bacteria representative of the deep fissure lesions of the cotton rat, bacteriological counts were made on whole teeth rather than on saliva or oral swabbings. This was done by extracting, weighing, and grinding the teeth, and making quantitative serial dilution counts. The counts were then calculated and recorded on the basis of numbers per gram of tooth.

Since it was found that molars from the lower jaw could not be extracted without fracturing the jaw, the molars from one of the upper quadrants were used. Arbitrarily, the upper right quadrant was chosen. Henceforth, reference made to extracted teeth applies to the three molars of the upper right quadrant.

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Immediately after the animal was killed, the teeth were extracted by means of a pair of sterile forceps and placed on a sterile watch glass in a sterile, previously weighed Petri dish. The teeth were then weighed and transferred to a sterile mortar by means of a few milliliters of sterile water from a 99 ml. water blank. Here they were ground with a sterile pestle until a uniform suspension was obtained. The entire suspension was then transferred aseptically to the 99 ml. sterile water blank, from which higher dilutions were made after thorough shaking.

To determine whether the majority of the microorganisms were located in the deep fissures or at the surface of the teeth, a series of determinations was made on the teeth from animals fed a ration (802) known to be highly cariogenic, and from animals fed the low caries-producing stock diet. The teeth from the upper right quadrant were extracted, weighed, and ground, and bacterial counts were made. The teeth from the upper left quadrant were also extracted and weighed, but were not ground. Instead, they were shaken with glass beads in a 99 ml. sterile water blank in order to remove any surface organism. Serial dilution counts were then made.

Freshly prepared media were used in all experiments. In the preparation of shake cultures used for quantitative purposes, diluted samples were placed into each of the five tubes containing approximately 10 ml. of melted agar at 45° C. and were thoroughly mixed with the agar. The tubes of inoculated solidified agar were incubated for three days at 37° C. At the end of this period the developing colonies were counted and total number per gram of tooth determined.

Since the bacteria associated with dental caries do not grow well in ordinary peptone media and require partially anaerobic conditions of culture, carrot-liver agar medium in shake tube cultures was used.

The culture medium used in these shake culture tubes was that of Garey, Foster, and Frazier,[9] and had the following composition:

Carrot extract	100 ml.	Washed agar	11 Gm.
Liver extract	100 ml.	Distilled water	800 ml.
Difco peptonized milk	10 Gm.	Sodium hydroxide to pH 7.0 ± 0.1	
Difco neopeptone	5 Gm.		

The carrot extract was prepared by adding one liter of water to one pound of ground carrots, and heating in a steamer for one hour. The extract was then filtered through cheesecloth, bottled, and sterilized in the autoclave. Likewise, the liver extract was prepared by adding one liter of water to one pound of ground fresh beef liver, heating to 70° C., and holding it at that temperature for thirty minutes. The resultant extract was filtered through cheesecloth, bottled, and sterilized in the autoclave.

In addition to the agar shake tube cultures, dilutions were also made in duplicate plates of Difco nutrient agar. This was done in order to obtain growth of strict aerobes present and thus give data for both aerobic and partially anaerobic conditions.

RESULTS AND DISCUSSION

The relationship of numbers of organisms found in ground and unground teeth from the same animal is shown in Table I and Fig. 1. These data represent animals fed the stock ration or the highly cariogenic ration (802). It may be seen that relatively few organisms were found in carious or caries-free

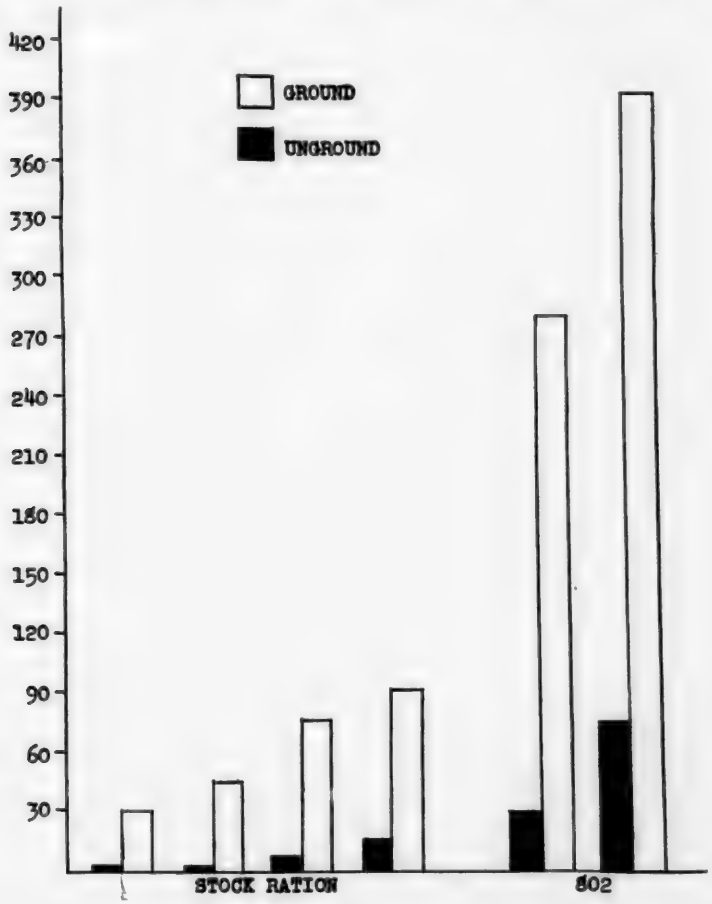

Fig. 1.—Relationship of numbers of organisms in ground and unground teeth. Number of organisms (1×10^6) per gram of tooth.

unground teeth, whereas much greater numbers of organisms were obtained from ground teeth. The difference in bacterial count was most marked when the incidence of caries was low. With the increase of incidence of caries the difference in bacterial count between ground and unground teeth narrowed, although a five- to tenfold increase in bacterial count still persisted in the ground teeth. From these data it is evident that bacterial counts from surface washing of teeth

of the cotton rat does not adequately portray the bacterial contamination present. It would appear that the bacteria are present in greatest numbers in the deep fissure cavities and are exposed most effectively by grinding. The noticeable narrowing of the count ratio between ground and unground carious teeth may indicate that heavily infected carious lesions by mere force of numbers of bacteria extrude organisms to the surface. The bacterial counts were uniformly higher in the shake tube cultures than in the nutrient agar plates. This would suggest that anaerobic bacteria are mainly responsible for the deep-fissure caries contamination.

TABLE I

NUMBERS OF ORGANISMS PER GRAM IN GROUND AND UNGROUND TEETH

RATION	GROUND TEETH		UNGROUND TEETH	
	SHAKE TUBE CULTURES	NUTRIENT AGAR PLATES	SHAKE TUBE CULTURES	NUTRIENT AGAR PLATES
Stock ration	33,840,000	6,384,000	1,515,000	545,000
	44,510,000	7,780,000	95,000	130,000
	64,280,000	5,178,000	3,004,000	1,094,000
	92,180,000	7,507,000	14,280,000	2,017,000
802	265,300,000	55,050,000	25,780,000	9,598,000
	362,200,000	26,590,000	69,530,000	12,020,000

Determinations made on ground and unground teeth of the cotton rat clearly indicate that the majority of the organisms are located in the deep fissures of the teeth; this is particularly evident in carious teeth where the relative numbers of organisms from ground teeth have been shown to be much greater than those from unground teeth. Since the majority of caries have been found to occur in the deep fissures, this large increase in numbers of organisms in ground carious teeth and relatively small increase in numbers of organisms in unground carious teeth is particularly significant.

SUMMARY

A method is described for determining the total bacterial count of carious teeth in the cotton rat. Bacterial counts made by this method show conclusively that the greater numbers of organisms in carious teeth are located in the deep-fissure lesions and may only be determined by grinding and culturing the entire tooth.

REFERENCES

1. Rosebury, T., Karshan, M., and Foley, G.: Studies of Lactobacillus in Relation to Caries in Rats, *J. D. Res.* **14**: 231, 1934.
2. Parsons, E., and Frobisher, M.: Effect of Dietary Carbohydrates on the Dental Flora of the Rat, *J. Bact.* **47**: 477, 1944.
3. Harrison, R. W.: Bacteriological Studies on Experimental Dental Caries in the Rat, *J. Infect. Dis.* **67**: 91, 97, 1940.
4. Harrison, R. W.: Bacteriological Studies on Experimental Dental Caries in the Rat, *J. Infect. Dis.* **67**: 106, 1940.
5. Orland, F. J.: The Oral Bacterial Flora as Related to Dental Caries in the Syrian Hamster, *J. D. Res.* **25**: 455, 1946.
6. Schweigert, B. S., Shaw, J. H., Phillips, P. H., and Elvehjem, C. A.: Dental Caries in the Cotton Rat. III. Effect of Different Dietary Carbohydrates on the Incidence and Extent of Dental Caries, *J. Nutrition* **29**: 405, 1945.

7. Schweigert, B. S., Shaw, J. H., Zepplin, M., and Elvehjem, C. A.: Dental Caries in the Cotton Rat. VI. The Effect of the Amount of Protein, Fat, and Carbohydrate in the Diet on the Incidence and Extent of Carious Lesions, *J. Nutrition* **31**: 439, 1946.
8. Schweigert, B. S., Potts, E., Shaw, J. H., Zepplin, M., and Phillips, P. H.: Dental Caries in the Cotton Rat. VIII. Further Studies on the Dietary Effects of Carbohydrate, Protein, and Fat on the Incidence and Extent of Carious Lesions, *J. Nutrition* **32**: 405, 1946.
9. Garey, J. C., Foster, E. M., and Frazier, W. C.: The Bacteriology of Brick Cheese. I. Growth and Activity of Starter Bacteria During Manufacture, *J. Dairy Sci.* **24**: 1015, 1941.

ENAMEL SOLUBILITY REDUCING EFFECT OF FLAVORED LOW CONCENTRATION STANNOUS FLUORIDE SOLUTION

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BIBBY,[1] Cheyne,[2] Knutson and Armstrong,[3] and others have shown that solutions of fluorine salts of sodium and potassium applied to the surfaces of the teeth will inhibit tooth decay. In all instances it was recommended that the teeth be carefully cleaned prior to the institution of a treatment series. For each treatment the mouth was divided into quadrants; each quadrant was isolated with cotton rolls for treatment. The isolated teeth were dried with air before application of the fluorine solution. Bibby[1] believed that further cleansing of the teeth with hydrogen peroxide followed by careful drying with alcohol was necessary. The fluorine solution was applied by rubbing the dried tooth surfaces with cotton saturated with the solution. Cheyne advocated a six-minute, Bibby recommended a seven-minute, and Knutson and Armstrong used a four-minute contact period for each segment of teeth isolated. Knutson and Armstrong showed that seven such contact periods were as effective as fifteen. Some form of these procedures now constitutes the conventional method for the topical application of fluoride for caries inhibition.

These procedures, although quite simple, are time-consuming and must be carried out by the dentist. If protective amounts of fluorine in solution could be applied to the teeth by the patient using a mouth rinse properly, the dentist's time could be devoted to other procedures. There is also a good possibility that more people would be benefited by a mouth rinse than could be treated by a dentist. Bibby[4] has already suggested the use of a mouthwash containing sodium fluoride, although when thirty-one adults (dental students) under his supervision used an acid pH 4.0 mouthwash containing 0.1 per cent sodium fluoride for one year, there was no reduction of caries activity below that of fifteen using a control fluoride-free mouthwash, or thirty-nine using no mouthwash.

Perry and Armstrong,[5] however, showed that the fluorine content of the enamel of mature erupted albino rat's teeth was increased by contact with drinking water containing fluorine. Volker[6] working with powdered enamel, demonstrated the fact that the enamel became less soluble when protected by solutions containing sodium fluoride. The rapidity with which fluorine solutions react with powdered enamel led him to believe that, during the process of drinking water, fluorine could be taken up, thereby reducing the solubility of the tissues and the incidence of caries. Dean[7, 8] corroborates this opinion by pointing out the fact that the upper anterior teeth are more completely protected by fluorine, for they have the greatest surface contact with the drinking water. In spite of the observations made by Bibby,[4] this finding that teeth in the oral cavity do pick up protective amounts of fluorine from the drinking water as it passes over them would indicate that fluoride solutions, if used long enough by young people, might be effective as a mouthwash.

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The flow of saliva will, of course, dilute the reagent solution in a mouthwash. Bossert and Dunning[9] have shown, however, that when a mouthwash consisting of a 0.1 per cent aqueous sodium fluoride solution is taken into the mouth and held for two minutes there is a dilution of only 0.0032 per cent.

The value of using a flavored, fluorine-containing solution as a mouth rinse for the inhibition of tooth decay should, of course, be tested clinically. However, the possibility that a flavored, low-concentration fluoride solution might reduce tooth decay can be quickly tested in the laboratory by using the method outlined by Volker,[6] Buonocore and Bibby,[10] or Muhler and Van Huysen.[11] This method, in brief, consists of comparing the solubility of unprotected and protected powdered enamel in a weak organic acid such as 0.2M acetic acid buffered to a pH of 4.0.

To make the laboratory test more closely conform to natural conditions, the powdered enamel used in this experiment and prepared by the Manly-Hodge separation method was covered with saliva in the following manner:

A one-gram sample of carbon tetrachloride dried powdered enamel which passed the 100 mesh but stayed on the 140 mesh sieve even with long agitation with a Kerr "vibrator" was stirred for sixty minutes in a beaker with 15 ml. of recently secreted saliva. It was then centrifuged, the saliva decanted, and the enamel dried with warm air at 40° C. This mixing with saliva and drying was done to make the separated enamel more like that found in the mouth where the surface of the tooth is always covered with this body fluid. This saliva-covered, air-dried powdered enamel was then divided into 100-milligram samples for the following studies: A. Solubility of air- and alcohol-dried unprotected powdered enamel. B. Solubility of air-dried powdered enamel protected by 1/500 water and flavored solutions of sodium and tin fluoride, respectively. C. Solubility of air-dried powdered enamel protected by 1/20,000 water and flavored solutions of sodium and tin fluoride, respectively.

A. Solubility of Saliva-Treated, Air- and Alcohol-Dried, Unprotected Enamel.—The first step was to compare with each other the solubility of 100 mg. of air-dried, and alcohol-dried, saliva-treated powdered enamel when subjected for a period of forty minutes to 25 ml. acetic acid adjusted with IN NaOH to a pH of 4.0.

Into each of two tared Selas crucibles there was placed 0.1000 Gm. of saliva-treated powdered enamel. One of these crucibles and its enamel content was then taken to a water suction system and washed with 25 ml. of 95 per cent alcohol. The contents of this crucible were filtered until dry.

Into each of the two crucibles, 25 ml. of the 0.2M acetic acid decalcifying solution were added and stirred for a period of forty minutes. Each crucible was then washed three times with exactly 5 ml. of distilled water. They were then taken to an oven and dried at 70° C. for six hours. At the end of this period, they were placed in a desiccator for six hours and weighed. They were repeatedly dried and desiccated until no greater variance than 0.0001-0.0002 Gm. was encountered. Twelve such tests were made, the data appearing in Table I.

During the combination of substances for the study of enamel solubility, it is necessary to determine the effect of the dry weights of the liquid reagents themselves upon the total weight changes which take place during the experiment. The necessity for this is very clearly brought out in a previous study[11] in which stannous fluoride combined with powdered enamel added so much weight to the 0.1000 Gm. of powdered enamel it protected, that subsequent to decalcification* it weighed more than 0.1000 gram. The effect upon the weight changes which take place during the combination of the alcohol and acetic acid with the powdered enamel brought about by the liquid volumes alone were examined by drying in separate tared Selas crucibles 5 and 25 ml. of alcohol and acetic acid, respectively. When this was done there was no weighable residue. It is, therefore, possible to point out that the weight changes in Section A of this experiment were brought about as a result of decalcification* only.

TABLE I

Comparison between enamel solubility reducing power of 1/20,000 flavored stannous fluoride solution and 1/5000 sodium fluoride in distilled water. It also shows the effect of washing saliva-covered powdered enamel and air-dried enamel with respect to solubility reducing power of fluoride solutions.

REAGENT	AIR-DRIED	ALCOHOL-WASHED
1. 25 ml. HaC	−0.0287	−0.0288
2. Distilled water 1/500 NaF	−0.0072	−0.0068
3. Distilled water 1/500 SnF_2	+0.0010	+0.0008
4. Flavored solution 1/500 NaF	−0.0065	−0.0062
5. Flavored solution 1/500 SnF_2	−0.0022	−0.0021
6. Flavored solution 1/20,000 NaF	−0.0099	−0.0100
7. Flavored solution 1/20,000 SnF_2	−0.0075	−0.0071

B. Solubility of Saliva-Treated, Air-Dried Enamel Protected by 1/500 Concentration Water and Flavored Solutions of Sodium and Stannous Fluoride, Respectively.—Into each of four tared Selas crucibles 100 mg. of saliva-treated, air-dried, powdered enamel were placed. The first of these crucibles was treated for twenty minutes with 5 ml. of 1/500 solution of sodium fluoride and water buffered with glacial acetic acid to a pH 4.0. The second of these crucibles was treated with 5 ml. of a 1/500 aqueous solution of stannous fluoride buffered with IN NaOH to a pH of 4.0. Into the third crucible there was placed 5 ml. of a buffered 1/500 flavored solution of sodium fluoride.† The fourth crucible

*A general tendency to call loss in weight decalcification seems prevalent from reading the literature. In some instances this may be exactly the csae. On the other hand, unless one measures the calcium and phosphorus that are removed from the enamel when it is subjected to an acidic solution, one cannot definitely say that loss of weight is a decalcification process.

†The formula for the flavored stannous fluoride solution is as follows:

Artificial flavor	gtt. IV
Tincture of thymol (5.0%)	gtt. I
Stannous fluoride	mg. XXV
Saccharin	gr. ss
Artificial coloring (2.5%)	gtt. V
Glacial acetic acid (U.S.P. XII)	gtt. VIII
Ethyl alcohol (95%)	ml. XXV
Distilled water q.s.ad.	500 ml.

contained, in addition to the 0.1000 Gm. of powdered enamel, 5 ml. of a buffered 1/500 concentration of flavored stannous fluoride solution. Each of these crucibles was then stirred for twenty minutes. The contents of the four crucibles were then washed and dried as previously described. The dried crucibles containing protected enamel then had added to them 20 ml. of 0.2M acetic acid decalcifying solution. These were stirred for a period of twenty minutes. Each crucible was again washed once with exactly 5 ml. of distilled water. They were dried in an oven at 70° C. for six hours and kept in a desiccator. They were reweighed after at least six hours' storage. When crucible weights varied more than 0.0001-0.0002 Gm., they were discarded. The data from twelve crucibles are averaged for use in Table I. Before arranging the data in the table, corrections were made when necessary for any weight added to the reactions by solution residues.

For the purpose of establishing in this part of the experiment which part of the weight changes contributed to the combination by the sodium fluoride and stannous fluoride reagents both in the unflavored and flavored solutions, the following control procedures were set up:

Five ml. of each of the respective 1/500 concentration of the stannous and sodium fluoride solutions in water flavored and unflavored were placed in a tared Selas crucible. These crucibles were attached to a water siphon apparatus and the liquid phase removed by slight suction. The crucibles were dried at 70° C. for six hours, placed in a desiccator for three hours, and then reweighed. From this procedure, it was found that there was a 0.0003 Gm. increase in weight when the 1/500 sodium fluoride in the aqueous solution was used. There was a 0.0028 Gm. increase when the 1/500 aqueous stannous fluoride solution was used. A 0.0010 Gm. increment occurred when the 1/500 flavored sodium fluoride solution was used and a 0.0036 Gm. increase when the 1/500 flavored stannous fluoride solution was used. Therefore, when these reagent solutions and enamel are combined there will be in all crucibles a weight increase that is equivalent to these values and must be deducted from the final weight. All values in lines 2, 3, 4, and 5 found in Table I have been corrected in this manner.

C. Solubility of Saliva-Treated, Air-Dried Enamel Protected by Water and Flavored Solution of Sodium and Stannous Fluoride, Respectively. Concentration, 1/20,000.—Into each of four tared Selas crucibles there was placed 100 mg. of saliva-treated, air-dried, powdered enamel. The first of the crucibles was treated with 5 ml. of buffered 1/20,000 sodium fluoride reagent made up in an aqueous solution. The second crucible was treated with a 1/20,000 aqueous solution of stannous fluoride. The third and fourth crucibles were treated with 1/20,000 flavored aqueous solutions of sodium fluoride and stannous fluoride, respectively. All four of the crucibles were then stirred for twenty minutes, following which the contents were filtered, and the remaining powdered enamel was washed three times with exactly 5 ml. of distilled water. Each crucible content was then combined with 20 ml. of pH 4.0, 0.2M acetic acid and stirred for twenty minutes. The crucible contents, following decalcification, were washed, dried, and weighed according to specifications previously described. In each of the instances, twelve such tests were made.

Control data to establish weight changes taking place because of reagents themselves were obtained and applied for correction of the experimental data. For control purposes, we again took a 5-ml. sample of prepared solution containing a 1/20,000 concentration of flavored sodium fluoride and placed it in a tared crucible. The liquid was removed by filtration and the crucible was then washed, dried, desiccated, and then reweighed. The residue for the flavored sodium fluoride solution was an average gain of 0.0004 Gm. Again this procedure was followed for the flavored 1/20,000 concentration of stannous fluoride. This residue weighed 0.0006 Gm. As in Section B, the values were deducted from the original experimental data before being recorded in Table I. Again controls were run for the distilled water, the actual solution, and the alcohol.

DISCUSSION

The data obtained in this study agree favorably with that obtained in another study,[11] namely, that saliva-covered powdered enamel when combined with 0.2M solution of acetic acid buffered to a pH of 4.0 loses presumably through decalcification about 28 per cent of its 100 milligrams. When powdered enamel is unprotected by saliva, it loses 29 per cent of its weight. Attempts to wash the protecting saliva residues from the enamel with alcohol do not seem to be effective.

As in other experiments,[11, 12] a 1/500 solution of sodium fluoride in water protects enamel so that only about 7 per cent of its weight is lost by decalcification. When stannous fluoride at 1/500 concentration is used, a weight gain instead of loss is brought about even after combination with acetic acid.

When the sodium fluoride is mixed with the flavored aqueous solution, it is more effective in reducing enamel solubility than when in the untreated aqueous solution. However, when stannous fluoride at 1/500 concentration is used in combination with a solution containing flavoring and preservative instead of an aqueous solution, the prepared solution interferes somewhat with its solubility protecting value. The stannous fluoride in this solution is, however, still better than any other protecting reagent solution.

It is interesting to note that a dilute solution of 1/20,000 concentration of sodium fluoride, or better yet, stannous fluoride, is relatively effective in reducing the solubility of saliva-covered enamel. Attempts to wash the saliva from the enamel before applying the protecting reagent in no way favors the protectiveness of the reagent against solubility.

If one may translate this information obtained from in vitro methods in the laboratory to the procedures used clinically, it is suggested here that washing teeth with alcohol before topical application of fluoride is of little value. It may also be suggested that the use of stannous fluoride in a mouthwash and at concentrations as low as 1/20,000 might be effective in reducing tooth decay.

Sodium fluoride at a concentration of 1/20,000 contains 22 ppm fluorine, whereas stannous fluoride has 12 ppm of this element. It is interesting to note, therefore, that stannous fluoride with about half the combined fluorine content of sodium fluoride is still more effective than the higher fluorine-containing sodium fluoride in reducing solubility.

REFERENCES

1. Bibby, B. G.: A New Approach to Caries Prophylaxis, *Tufts D. Outlook* **15**: 4, 1942.
2. Cheyne, V. D.: Human Dental Caries and Topically Applied Fluorine: A Preliminary Report, *J. A. D. A.* **29**: 804, 1942.
3. Knutson, J. W., and Armstrong, W. D.: Value of Topically Applied Sodium Fluoride in the Control of Dental Caries, *Pub. Health Rep.* **58**: 1701-1715, 1943.
4. Bibby, B. G.: Preliminary Reports on the Effect on Dental Caries of the Use of Sodium Fluoride in a Prophylactic Cleaning Mixture and in a Mouth Wash, *J. D. Res.* **25**: 207, 1946.
5. Perry, M. W., and Armstrong, W. D.: On the Manner of Acquisition of Fluorine by Mature Teeth, *J. Nutrition* **21**: 35, 1941.
6. Volker, J. F.: Effect of Fluorine on Solubility of Enamel and Dentin, *Proc. Soc. Exper. Biol. & Med.* **42**: 725-727, 1939.
7. Dean, H. T., Jay, P., Arnold, F. A., Jr., and Elvove, E.: Domestic Water and Dental Caries, *Pub. Health Rep.* **56**: 761-792, 1941.
8. Dean, H. T., Arnold, F. A., Jr., and Elvove, E.: Domestic Water and Dental Caries, *Pub. Health Rep.* **57**: 1155-1179, 1942.
9. Bossert, W. A., and Dunning, J. M.: Salivary Dilution of 1-1000 Sodium Fluoride Solution Used as a Mouthwash, *J. D. Res.* **24**: 311, 1945.
10. Buonocore, M. G., and Bibby, B. G.: The Effect of Various Ions on Enamel Solubility, *J. D. Res.* **24**: 103-109, 1945.
11. Muhler, J. C., and Van Huysen, G.: Solubility of Enamel Protected by Sodium Fluoride and Other Compounds, *J. D. Res.* **26**: 119-127, 1947.
12. Phillips, R. W., and Muhler, J. C.: Solubility of Enamel as Affected by Fluorides of Varying pH, *J. D. Res.* **26**: 109-117, 1947.

THE EFFECT OF VARIOUS INORGANIC SALTS ON THE SOLUBILITY OF CALCIUM PHOSPHATE, TOOTH ENAMEL, AND WHOLE TEETH IN LACTIC ACID

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SINCE our previous publication[1] further evidence in support of the mechanism proposed for fluorine action on teeth has been gained from experiments with barium nitrate, silver nitrate, silver fluoride, and lead nitrate. All of these materials exhibited a "protective effect" on whole teeth suspended in lactic acid, and all reduced the "apparent solubility" of tooth enamel and tricalcium phosphate in lactic acid. In the case of lead nitrate and silver fluoride, increases instead of decreases in the weight of residue were obtained, no doubt as a result of precipitation of lead phosphate and silver phosphate, both of which have greater molecular weights than the calcium phosphate being dissolved.

The suggestion made at that time to account for the protective effect of fluorine, i.e., that the tooth enamel is dissolved in lactic acid and then the calcium reprecipitated as calcium fluoride, must now be widened so as to include any material which will precipitate insoluble calcium salts such as oxalates and, in addition, any material which will precipitate insoluble phosphates, such as barium, lead, and silver salts.

A summary of the results obtained with these salts is given in Table I. The numbers in brackets refer to values obtained in control tubes.

TABLE I
THE EFFECT OF VARIOUS SALTS ON THE DECALCIFICATION OF TEETH, AND ON THE SOLUBILITY OF TOOTH ENAMEL AND TRICALCIUM PHOSPHATE IN 1 PER CENT LACTIC ACID

MATERIAL ADDED	WHOLE TEETH		TOOTH ENAMEL		TRICALCIUM PHOSPHATE		
	MG. P PER 10 C.C.	MG. Ca PER 10 C.C.	CHANGE IN WEIGHT MG.	MG. P PER 10 C.C.	CHANGE IN WEIGHT MG.	MG. P PER 10 C.C.	MG. Ca PER 10 C.C.
NaF	2.83	7.96	−25	3.34	−50	5.9	9.0
0.01M	(9.5)	(23.2)	(−50)	(5.6)	(−80)	(6.0)	(30.6)
$(NH_4)_2C_2O_4$	1.22	–	−36	7.0	−22	9.8	–
0.01M	(5.6)	–	(−65)	(5.0)	(−68)	11.6	–
$Pb(NO_3)_2$	0.78	3.83	+10	0.27	+66	0	11.9
0.05M	(5.3)	(25.9)	(−38.2)	(5.7)	(−67)	(8.72)	(28.5)
$Ba(NO_3)_2$	3.42	11.21	−16.2	2.40	−10.2	10.3	27.2
0.05M	(5.3)	(25.9)	(−38.2)	(5.7)	(−62)	(11.6)	(26.8)
$Ag(NO_3)_2$	2.40	12.85	−12.1	2.11	−13.5	9.8	31.4
0.05M	(5.3)	(25.9)	(−38.2)	(5.7)	(−62)	(11.6)	(26.8)
AgF	2.41	7.40	+8	7.5	+24	4.32	7.9
0.01M	(9.5)	(23.2)	(−50)	(5.6)	(−67)	(8.72)	(28.5)

In the experiments with barium nitrate there was a slight trace of decalcification.

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Concentrated solutions of calcium salts and phosphates, when used in similar experiments, reduced the real solubility of tooth enamel as shown by phosphate analysis, but had little, if any, effect on the weight of residue in the tubes. Solutions containing 16 mg. per cent Ca and 10 mg. per cent P had a protective effect on teeth.

These results support our previous contention that any salt whose anions form an insoluble calcium salt (e.g., ammonium oxalate) or whose cations form insoluble phosphate salts (e.g., lead nitrate, barium nitrate) will protect the teeth and reduce the apparent solubility of tooth enamel and calcium phosphate.

REFERENCE

1. Rae, J. J., and Clegg, C. T.: *J. D. Res.* **24**: 235, 1945.

CHANGES IN THE CALCIUM AND PHOSPHATE CONCENTRATIONS OF SALIVA AND INORGANIC SALT SOLUTIONS ON SHAKING WITH CALCIUM PHOSPHATE

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SALIVA from caries-susceptible and caries-free individuals shows few chemical differences, and it would be an extremely useful clinical test if some decisive criterion to distinguish between these two types of saliva were available.

Karshan[1, 2] has stated that although the mg. per cent calcium and mg. per cent phosphorus are the same in saliva from both caries-free and caries-active individuals, the change in the concentration of these ions produced by shaking the saliva with excess powdered tricalcium phosphate is remarkably different. In stimulated saliva from caries-free individuals, 65 per cent of the calcium and 20 per cent of the phosphate were removed by shaking with tricalcium phosphate, whereas saliva from caries-immune individuals had 39 per cent of the calcium and 45 per cent of the phosphate removed by the same treatment.

These surprising results prompted us to investigate this phenomenon further in an attempt to gain some insight into the chemical processes involved.

On shaking saliva with tricalcium phosphate (Kahlbaum's reagent) we were unable to observe any decrease in phosphate but did obtain varying decreases in the calcium concentration.

When an aqueous solution of calcium nitrate and sodium phosphate of approximately the same calcium and phosphorus concentrations as saliva was used, an analogous effect was observed, viz., an increase in phosphate and a decrease in calcium (Table I). The final pH of the solutions was approximately 5.60.

TABLE I

THE EFFECT OF SHAKING WITH TRICALCIUM PHOSPHATE ON THE CALCIUM AND PHOSPHATE CONCENTRATIONS OF SOLUTIONS

SOLUTIONS USED	BEFORE SHAKING		AFTER SHAKING		CHANGE PRODUCED	
	P mg. %	Ca mg. %	P mg. %	Ca mg. %	mg. % P	mg. % Ca
Saliva	13.0	9.75	31.4	2.89	+18.4	−6.86
Calcium nitrate and sodium phosphate solution	12.6	8.4	18.2	2.6	+5.6	−5.8

Karshan has suggested that the ratio of calcium to phosphorus in the calcium phosphate used has an important bearing on the results obtained. To investigate this possibility we prepared a series of calcium phosphate mixtures

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made from calcium triphosphate and calcium diphosphate and shook these with solutions of calcium and phosphate approximating the composition of saliva. The Ca/P ratio of the Kahlbaum's tricalcium phosphate was found to be 2.10 and of the Kahlbaum's calcium diphosphate, 1.45 (Table II). The initial composition of the solution was 8.42 mg. per cent calcium and 13.1 mg. per cent phosphorus.

TABLE II

THE EFFECT OF SHAKING WITH TRICALCIUM PHOSPHATE ON THE CALCIUM AND PHOSPHATE CONCENTRATIONS

WEIGHT RATIO CA/P OF SOLID USED	CALCIUM		PHOSPHATES	
	FINAL MG. %	CHANGE PRODUCED MG. %	FINAL MG. %	CHANGE PRODUCED MG. %
1.45	2.85	−5.57	11.7	−1.4
1.59	2.96	−5.46	22.0	+8.9
1.76	3.60	−4.82	26.4	+13.3
2.10	2.15	−6.27	21.8	+8.7

These results indicate that in all cases except for calcium diphosphate (Ca/P = 1.45) the phosphorus rose to about twice its original value and the calcium decreased to 40 per cent of its original value. With the exception of calcium diphosphate, changes in Ca/P ratio seem to have little effect on the results. Even with calcium diphosphate the change in phosphorus produced was almost within experimental error.

Another possible explanation of the results shown in Table I (i.e., an increase in phosphate and a decrease in calcium) may be found in the pH of the resulting solutions. For example, on shaking the calcium phosphate and calcium diphosphate with sodium acetate-sodium veronal buffer solutions at different pH's, the results given in Table III were obtained.

TABLE III

THE EFFECT OF pH ON THE SOLUBILITY OF TRICALCIUM PHOSPHATE AND DICALCIUM PHOSPHATE IN BUFFERED SOLUTIONS

pH		TRICALCIUM PHOSPHATE $Ca_3(PO_4)_2$			pH		CALCIUM HYDROGEN PHOSPHATE $CaHPO_4$		
INITIAL	FINAL	Ca MG. %	P MG. %	Ca/P BY WEIGHT	INITIAL	FINAL	Ca MG. %	P MG. %	Ca/P BY WEIGHT
4.50	5.50	33.3	19.6	1.70	4.60	6.47	38.0	26.0	1.46
5.05	5.28	16.6	13.9	1.19	5.24	6.72	19.5	13.0	1.50
5.70	5.53	9.6	10.6	0.91	5.90	7.10	7.6	7.0	1.08
7.48	6.59	1.1	12.3	0.089	7.46	7.62	3.6	6.2	0.61
8.40	7.15	0.55	13.9	0.004	8.22	7.72	1.6	15.7	0.09

The fact that these buffer solutions, when shaken with calcium phosphates, do not yield solutions with theoretical Ca/P ratios [$Ca_3(PO_4)_2$ = 1.94 and $CaHPO_4$ = 1.29] suggests that the CaO and P_2O_5 components of these compounds dissolve independently, and that the ratio of Ca/P obtained and the amounts of calcium and phosphate dissolved depend upon the final pH of the solution. The amounts of calcium dissolved in both these experiments decreased

with increasing pH. The amount of phosphate dissolved passed through a minimum between pH 5 and 7.

The results of shaking solutions containing calcium and phosphate with tricalcium phosphate at varying pH's showed that at a final pH of 4.95 both the Ca and P increased, but at a final pH of 7.60 the Ca dropped and the P increased (Table IV).

TABLE IV

THE EFFECT OF PH ON SHAKING SOLUTIONS CONTAINING CALCIUM AND PHOSPHATE WITH TRICALCIUM PHOSPHATE

pH		CALCIUM MG. %		PHOSPHORUS MG. %	
INITIAL	FINAL	INITIAL	FINAL	INITIAL	FINAL
4.52	4.95	8.04	41.1	16.5	36.0
5.20	5.25	8.04	24.4	16.5	34.4
6.65	5.78	8.04	9.26	16.5	30.0
7.40	6.32	8.04	2.19	16.5	25.5
8.50	7.60	8.04	0.84	16.5	27.0

When the pH of saliva was adjusted with HCl or NH_4OH, and then shaken with tricalcium phosphate, the results shown in Table V were obtained.

TABLE V

THE EFFECT OF PH ON THE CHANGES IN CALCIUM AND PHOSPHORUS PRODUCED BY SHAKING SALIVA WITH TRICALCIUM PHOSPHATE

pH		CALCIUM MG. %		PHOSPHORUS MG. %	
INITIAL	FINAL	INITIAL	FINAL	INITIAL	FINAL
3.97	4.75	11.76	35.2	10.6	40.5
5.60	5.38	11.76	11.3	10.6	30.0
7.40	6.30	11.76	2.22	10.6	34.5

These results show that at a final pH of 4.75 the calcium and the phosphate values both increased on shaking with calcium phosphate, whereas at a higher final pH (6.30) the calcium decreased and the phosphate increased.

SUMMARY AND CONCLUSIONS

Saliva and inorganic salt solutions containing comparable amounts of calcium and phosphate, when shaken with tricalcium phosphate, yield solutions with higher phosphate concentrations and lower calcium concentrations than the original solutions.

When buffers, ranging in pH from 5.5 to 7.15, were shaken with tricalcium phosphate, the amounts of calcium and phosphate dissolved had no apparent relation to the amounts of calcium and phosphate in the solid. The calcium values obtained decreased continuously with increasing pH; the phosphate passed through a minimum at pH 5.5.

Inorganic salt solutions containing calcium and phosphate, when shaken with calcium phosphate, showed an increase in both calcium and phosphate at a low pH, but at a higher pH the calcium decreased and the phosphate increased. Analogous results were obtained with saliva.

These experiments lead us to conclude that variations in concentration of calcium and phosphate of salivas, produced by shaking these salivas with calcium phosphate, bear little if any relation to the Ca/P ratio of the calcium phosphate used. The pH of the saliva or solutions used seems to be the deciding factor in determining whether the calcium concentration increases or decreases on shaking with calcium phosphate.

REFERENCES

1. Karshan, M.: *J. D. Res.* **15**: 383, 1936.
2. Karshan, M.: *J. D. Res.* **18**: 395, 1939.

THE VALUE OF THE ROENTGENOGRAPH IN THE DETECTION OF CARIOUS LESIONS

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THE widespread use of the roentgenograph in detecting carious lesions of the teeth demonstrates its value in supplementing clinical procedures. The extent to which various examiners depend upon this means of diagnosis, however, varies greatly. In a report by Day and Sedwick[1] on a mixed group of 433 children whose average age was 13 years, 5 per cent of the total number of cavities would have been overlooked had the roentgenograph not been used. In an examination of 952 freshmen in a large university by Delabarre,[2] 51 per cent of the cavities were found only through roentgenographic examination. Fixott,[3] in reporting on the value of roentgen rays in children's dentistry, stated that from 25 to 40 per cent more dental caries can be detected than by the single clinical procedure employing mouth mirror, explorer, and visual inspection. White,[4] utilizing a single posterior bite-wing film for each side of the mouth, located 4.4 carious areas per patient in an examination of 256 cadets. In 56 per cent of the patients, 1.4 carious areas per individual required immediate attention. Although Smith[5] did not discuss the influence which the roentgenograph had on the total caries score, in his study of 210 children, ages 3 to 12 years, two-thirds of all patients showed an addition of from one to nine cavities.

The present study was undertaken because a considerable amount of data has been published in connection with dental caries investigations at the University of Iowa during the past twenty years without mentioning the role the roentgenograph plays in the figures presented on various groups studied. It is a routine policy to take at least bite-wing roentgenographs on all clinical patients. Patients delegated for special study have full-mouth roentgenographic surveys taken at the time of each examination if the interval between visits exceeds six months. As a rule, so few additional cavities are found by either method following the type of meticulous clinical examination performed in our clinics that it has been the opinion of reporters that the relative incidence of newly found cavities need not be discussed in detail as influencing the expressions of carious involvements. Proof of this assumption, which is the principal objective of the present study, is offered by analysis of the data.

Because many of our children receive constant and complete dental care, the second objective of the study was to estimate the value of the roentgenograph as a diagnostic aid in daily efforts to preserve teeth through local care. The latter factor was considered important because it is well known that early de-

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tection of dental caries is the most important step in a program of tooth conservation. The finding of approximal caries is of unusual value in this regard. Other than in this type of cavity, however, there is seldom much need for the roentgenograph in supplementing clinical information, at least in matters which might affect a group rating.

The present report is not concerned with a classification of roentgenographic findings into occlusal, proximal, or recurrence of decay insofar as other published material adds the relative proportions found by inspections. The highest percentage of accuracy in detecting these types is still the clinical method of examination. The combination giving the highest percentage of accuracy in diagnosing proximal and recurrent caries is that of the clinical and bite-wing inspections.[6, 7] We have not attempted to discuss the use of the roentgenograph in detecting missing teeth, supernumerary teeth, tumors and cysts, bone and tooth growth, resorption, and physiologic age of the child's dentition.

PROCEDURE

A brief résumé of the type of clinical examination in current usage in the pedodontic clinics at the University of Iowa has been presented by Boyd and Cheyne.[8] From fifteen to twenty-five minutes are required to complete the clinical phases of the procedure. Carious surface recordings are made on all deciduous and permanent teeth in the mouth, and note is made as to the presence or absence of each tooth. Missing deciduous teeth are not taken into account because it is difficult to determine the physiologic dental age of each subject. Such teeth, therefore, are assumed to have been shed rather than lost because of decay. This latter item is insignificant in the groups studied, insofar as it is known that patients receiving constant dental care lose few teeth prematurely.[8] If such teeth had been counted, this would have depressed the roentgenographic percentage of findings still more. For permanent teeth, affected tooth surfaces have included those which are decayed, missing through extraction, or filled. Extracted teeth are assumed to have had three decayed surfaces prior to extraction. The symbol DMF is used to express the incidence of present and past caries, implying that the teeth are either decayed, missing, or filled. The symbol DF is more applicable to deciduous teeth. The evaluation of caries on the basis of tooth surfaces as presented in this paper is merely a refinement of the DMF.

Four groups of children were selected for comparative studies in order to evaluate the role of the roentgenograph in detecting carious lesions under various conditions. The first two groups were made up of children studied in connection with investigations which are in progress in a state school for feebleminded children at Glenwood. At the time of the initial examination three full-time examiners leisurely inspected approximately 300 children in detail. From the number it was possible to select 197 individuals on whom bite-wing roentgenographs could be taken. Re-examination six months later permitted thirty-one additional examinations to be supplemented by bite-wing roentgenographs. Because the latter examinations were more hurried, it was decided to include these children as a group for comparison with those examined at the time of the

first trip. Considered collectively, the Glenwood children comprise a group with a relatively low caries score and are used for comparison with normal children receiving bite-wing and full-mouth pictures.

All roentgenographs were taken by registered nurses or technicians especially trained in the procedure and working under the supervision of a dentist. Two posterior bite-wing films were taken on patients receiving this type of inspection; for those receiving the full-mouth surveys, from ten to fourteen exposures were made. The number and size of the films varied according to the age of the child. Bite-wing films were not taken of the anterior region routinely in our clinics because most of the cavities in this region are revealed by the clinical method of examination. Bodmer[6] confirms this observation. All films were of acceptable density such as are obtained under routine conditions, and included the areas specified. If the teeth were not clearly represented, new exposures were made until the final pictures were as accurate as conditions permitted. They were read by a dentist using a fluorescent illuminator; the cavities were added to the clinical form with a colored pencil which differentiated them from the clinical findings.

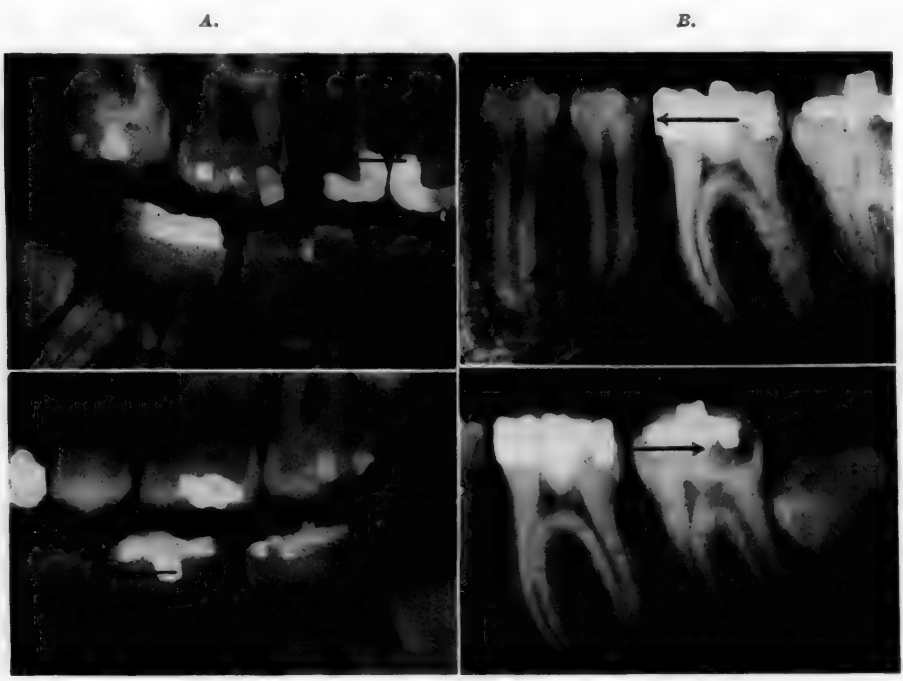

Fig. 1.—*A*, dentinal caries; *B*, enamel etching; *C*, questionable area in enamel; D, recurrence of caries under filling.

Three types of carious lesions were noted in the roentgenograph, namely: (1) dentin involvements, (2) enamel etchings, without penetration into the dentin, and (3) questionable areas (Fig. 1, *A*, *B*, and *C*). Sometimes it was diffi-

cult to determine whether one was viewing a decavitation, hypoplastic area, artifact, or a thinner portion of enamel, especially as the latter occurs in the cervical region of a tooth; but if any area resembled a cavity it was recorded in one of the three categories. Recurrence or remaining decay under fillings was counted as roentgenographic findings of a dentinal nature if detected by this method (Fig. 1, *D*).

TABLE I

DISTRIBUTION OF 429 CHILDREN ACCORDING TO AGE AND SEX

AGE GROUP	A. BITE-WING FILMS						B. FULL-MOUTH SURVEYS				
	GLENWOOD TRIP 1		GLENWOOD TRIP 2		DENTAL CLINIC		DENTAL CLINIC		TOTAL		
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	ALL
3- 5 yrs.	0	0	0	0	0	1	2	2	2	3	5
5- 7 yrs.	5	3	0	0	0	0	11	7	16	10	26
7- 9 yrs.	5	6	1	0	7	14	10	15	23	35	58
9-11 yrs.	18	15	0	0	15	9	6	7	39	31	70
11-13 yrs.	12	13	1	1	22	12	12	9	47	35	82
13-15 yrs.	21	12	5	1	14	5	9	10	49	28	77
15-17 yrs.	26	17	8	3	2	0	0	0	36	20	56
17-19 yrs.	17	27	11	0	0	0	0	0	28	27	55
Total	104	93	26	5	60	41	50	50	240	189	429

PRESENTATION OF MATERIAL

The average age of the children receiving bite-wing roentgenographs was 159 months; those receiving full-mouth surveys, 117 months. The youngest child on whom data are presented was 36 months; the oldest, 234 months. The distribution of all children examined according to age and sex is presented in Table I. A total of 240 male patients and 189 females was studied. A total of 658 bite-wing roentgenographs was inspected on patients seen at Glenwood and the dental school. There were 1,036 single films taken in connection with the full-mouth surveys.

Table II presents detailed data of the roentgenographic findings on all individuals studied at Glenwood and the dental school. In Section A of the table an analysis is given of the subjects on whom bite-wing films were studied; in Section B a résumé of the material found by inspection of the full-mouth surveys is presented. It is of interest to note that, of the patients on whom bite-wing pictures were taken, 234 of the total 4,575 carious surfaces were found in the roentgenograph. In the group receiving full-mouth surveys, 132 of the 2,165 involved surfaces were found in the roentgenograph. Statistical evaluation of the two groups regarding the manner in which cavities found by the roentgenograph affected the individual clinical scores of 13.9 and 21.7 DMF tooth surfaces showed there was no significant change. A comparison of the two groups of Glenwood children shows similar average DMF tooth surfaces per child. Although the examination of the second group was relatively hurried, actually a smaller number of cavities per individual was revealed roentgenographically, i.e., 0.74 cavity per child contrasted to 0.23 for the first group. One explanation of this might be that the individuals in the second group were more cooperative,

because considerable rejection of undesirables had been done at the time of the first examination. Statistical comparisons between these two groups are impossible because of the small sample represented in the second group.

TABLE 11
ANALYSIS OF ROENTGENOGRAPHIC FINDINGS ON THE BASIS OF SUBJECT PERSONNEL

A. BITE-WING FILMS

	GLEN-WOOD TRIP 1	GLEN-WOOD TRIP 2	DENTAL CLINIC	TOTAL BOYS	TOTAL GIRLS	TOTAL ALL
Number of subjects	197	31	101	190	139	329
Subjects with no additional cavities shown by roentgenographs	129	27	53	123	86	209
DMF surfaces: total	2102	349	2124	2587	1988	4575
Average per child	10.7	11.3	21.0	13.6	14.3	13.9
DMF surfaces found in roentgenographs	146	7	81	118	116	234
Dentin	34	6	37	46	31	77
Enamel	104	0	27	54	77	131
Questionable	8	1	17	18	8	26
Percentage of total	6.9%	2.0%	3.8%	4.6%	5.8%	5.1%
Average per child: dentin	0.17	0.19	0.37	0.24	0.22	0.23
Average per child: enamel	0.53	0.00	0.27	0.28	0.55	0.40
Average per child, total	0.74	0.23	0.80	0.62	0.83	0.71

B. FULL-MOUTH SURVEYS
(DENTAL CLINIC)

	BOYS	GIRLS	ALL
Number of subjects	50	50	100
Subjects with no additional cavities shown by roentgenographs	22	22	44
DMF surfaces: total	1094	1071	2165
Average per child	21.9	21.4	21.7
DMF surfaces found in roentgenographs	64	68	132
Dentin	31	36	67
Enamel	12	12	24
Questionable	21	20	41
Percentage of total	5.9%	6.3%	6.1%
Average per child: dentin	0.62	0.72	0.67
Average per child: enamel	0.24	0.24	0.24
Average per child, total	1.28	1.36	1.32

The 101 children from the dental school whose bite-wing roentgenographs were examined demonstrate a group with a relatively high number of DMF tooth surfaces. There is approximately twice the amount of caries for the group as compared with the average of the Glenwood children. In such a comparison it might be logical to assume that the group showing the highest number of DMF tooth surfaces clinically also would show a higher percentage of lesions in the roentgenographs, but this was not the case. There was a greater proportion of enamel lesions, however, in the group with less caries. One might interpret this by suggesting that, not only were cavities fewer in number in the less susceptible group, but those that were present did not involve dentin as rapidly.

An analysis of the data concerned with the findings in the full-mouth surveys of 100 children demonstrates no tendency for girls to develop more caries than boys. There is an equal distribution of the cases on the basis of sex and an equal number of caries-free individuals in each group. Sex, therefore, is

not a factor in calculations which take into account caries-immune, average DMF tooth surfaces, or the number of cavities found by roentgenograph in the dentin and enamel. Similar deductions can be made with regard to the summarized data on sex using the bite-wing films. Nineteen boys and girls had only one additional cavity found in the apical films; one boy demonstrated seven additional cavities; one girl demonstrated six additional cavities. Although the percentage of cavities found in the full-mouth study was nearly the same as that found by the use of bite-wing films alone, the individual roentgenographic findings per child were nearly doubled. The significance of this statement is lost, however, if one considers that the full-mouth survey group had an average higher caries rating and that a more logical evaluation of the manner in which full-mouth surveys influence the caries rating is made by comparing this group with a similar dental clinic group receiving bite-wing films. When this is done,

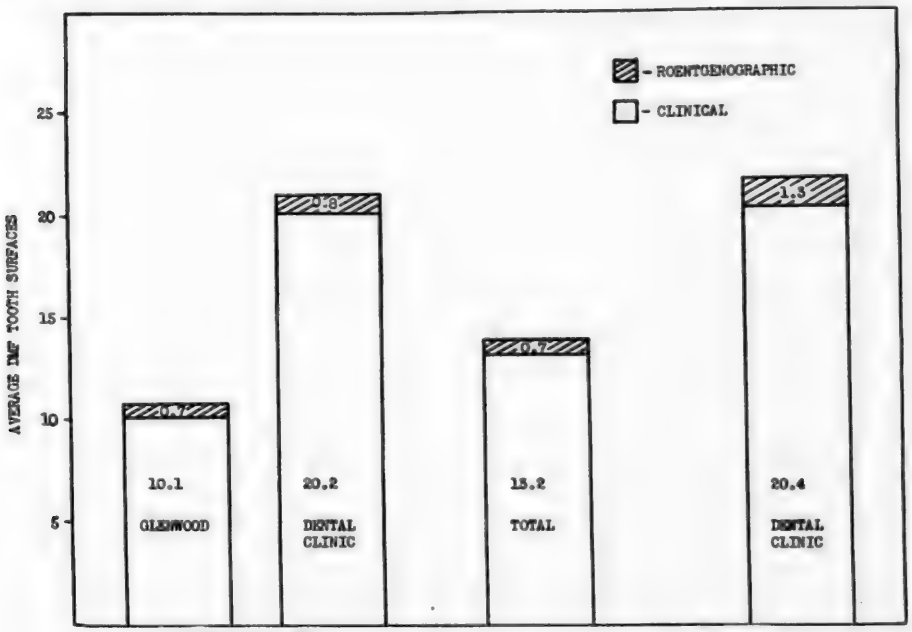

Fig. 2.

the individual caries pattern revealed by the full-mouth survey denotes a marked increase. Fig. 2 is shown to emphasize the differences in average DMF tooth surfaces for the compared groups, as well as to reveal the relative proportion of roentgenographic findings using bite-wing films and full-mouth surveys. A greater number of children revealed at least one cavity using the full-mouth survey contrasted to the individuals on whom bite-wing roentgenographs were taken, i.e., 56 per cent compared with 36 per cent (Fig. 3). The need for full-mouth surveys is thus indicated if one tries to find a greater number of cavities.

Based upon the number of surfaces shown, the bite-wing roentgenograph is better than the regular apical film for detecting interproximal caries. Undoubtedly, this difference is due to the slighter degree of angulation of the roentgen cone projected against the crown when bite-wing films are used. A relatively higher number of questionable lesions are found when only the apical film is used, whereas with the bite-wing film these questionable areas fall into the definite classification of an enamel or dentin cavity, i.e., 208:26 compared to 91:41 (Fig. 3).

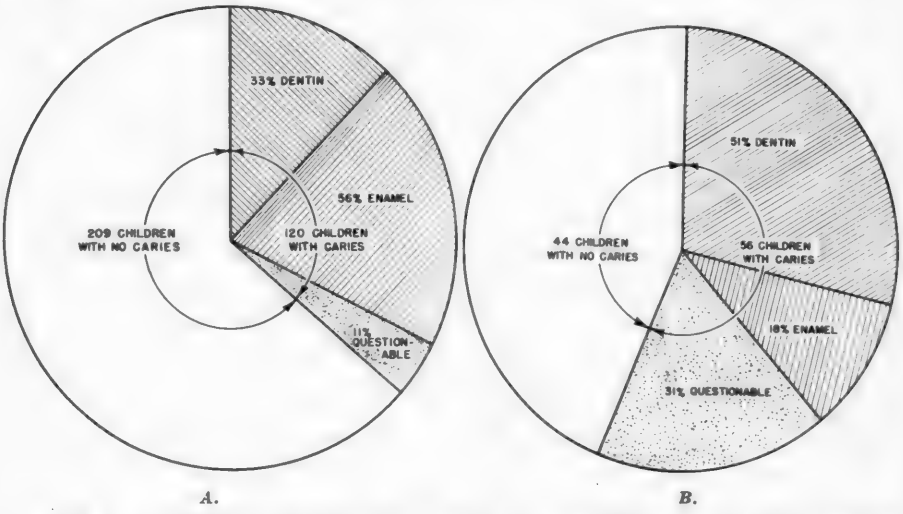

Fig. 3.—*A*. Bite-wing film percentage chart. The unshaded area shows the percentage of cases which had no additional cavities found by roentgenograph; the shaded areas, the percentage which had lesions, broken into dentin, enamel, and questionable in type. *B*. Full-mouth survey percentage chart. The data on this group are presented in a similar manner.

All of the children receiving full-mouth surveys were regular patients in the dental clinic. Some had been coming for years and were regularly inspected. Because one might assume that successive examinations on the same individual would reveal less caries in the roentgenograph, the children were arranged on the basis of the number of times they had been examined. After this was done, however, there was no indication that less caries was revealed per child as the number of examinations increased. Likewise, when the children were rearranged according to age, in order to determine the number of cavities revealed at various levels, no consistent variations could be obtained. This confirms the findings of Barr,[9] who presented data on age groups ranging from 5 to 13 years. An interesting observation in this regard was that two boys and two girls in the 3- to 5-year-old range had a total of seventy-four surfaces found to be affected by clinical methods, with an additional six involvements found in the roentgenograph.

DISCUSSION

Cohen,[10] in reporting on the susceptibility of young diabetics to dental caries, found that there is an accelerated rate of acquiring cavities with

advancing age and concluded that diabetics do not have a greater immunity than nondiabetics. This is in contrast to reports by Boyd and Drain,[11] in which caries advance has been shown to be markedly inhibited with improvement of the diet. Although the number of lesions found by Cohen in the roentgenograph is not stated in his report, the opinion is expressed that detailed roentgenographic examinations probably accounted for the results. Roentgenographic findings are considered a part of the general caries figures in the literature on diabetic subjects studied in our clinics. Moreover, deducting the average number of lesions found in the roentgenographs does not disturb the general trend which our data show.

It is possible to find figures in the literature which states that from 5 to over 50 per cent of cavities would be overlooked if the roentgenograph were not used. The reason sometimes given is that clinical examinations on children must be made under such adverse circumstances that roentgenographs offer the only dependable means of diagnosis. At Glenwood some of the patients were not sufficiently capable mentally to appreciate the technique of roentgenography, although they sat through the clinical examination with ease. Moreover, we have not experienced this difficulty in normal patients accepted for operative procedures in our clinics. With a little patience, even the youngest child becomes adjusted to a roentgenographic examination.

In reviewing the roentgenographs preparatory to adding the findings to the clinical record, it was found in our study that some proximal cavities that could not be seen in the film picture could be found by digital and visual examination. Undoubtedly this condition was due to overlap or to lack of proper density of the film in the region, or, as mentioned by McCall and Wald,[12] breaks in the enamel might have been overshadowed by unusual density of the structure itself. Similarly, questionable or shallow etchings appearing on the roentgenograph frequently are found to be deep clinically. This is particularly true when first permanent molars are involved. Slight buccal or lingual version of the teeth, or differences in position of those in the maxilla as compared to those in the mandible, may cause the same phenomenon when bite-wing films are used. In contrast, some areas thought to be carious in the roentgenograph were found to be negative with the explorer tine. Many cavities suspected clinically were verified by the roentgenograph. These were not counted as cavities found in the latter method. Arnett and Ennis[13] found 1,662 carious teeth in a group of college students by clinical examination, and 1,372 by complete intraoral roentgenographic examination, but the two methods were in agreement for only 237 of the teeth.

A brief summary of controversial points encountered in determining the value of the roentgenographic findings in dental caries presented by Barr[9] on a group of 157 children with a total of 117 proximal cavities is interesting. He states: "Of these, 626 or 56.04 per cent were evident on clinical examination, and 1,040 or 93.11 per cent were by radiographic examination. As 549 (49.15 per cent) of the total cavities diagnosed were shown by both methods of examination, it will be seen that no less than 491 cavities or 43.96 per cent were not

detected during clinical examination, i.e., were shown only by radiographic examination. A much smaller number—77 or 6.89 per cent—were not evidenced by the radiographic examination, i.e., were found only by clinical examination.''

Undoubtedly a large part of our success in detecting proximal lesions is due to the fact that they are carefully and systematically sought by means of the No. 2 S. S. White explorer point and good illumination. Opacity of the marginal ridge area is a place to inspect. We do not use transillumination which, undoubtedly, would be of assistance. This should supplement a thorough clinical examination of the teeth, in a cleansed condition and free from debris, a condition which has been shown by Sognnaes[7] to increase the total number of lesions found by clinical methods 20 per cent.

There is no 100 per cent effective method for finding all caries in a mouth. Perfection can be approached by a combination of various methods, but no one can know the total caries picture of an individual. It is quite possible that some cavities can be overlooked by a combination of all methods of examination. Burket[14] has shown that 24 per cent of cavities in teeth previously examined by clinical and roentgenographic methods of examination could be demonstrated only in microscopic preparations. Undoubtedly this procedure approaches perfection, but it is impossible to utilize the microscope in the clinical search for caries.

SUMMARY

1. A detailed clinical method of examination for caries on 429 children has been checked for accuracy by arranging the individuals into different groups, according to varying caries incidence and examination conditions, using the bite-wing film and full-mouth serial roentgenographs. From the data presented it is possible to state that neither a clinical nor roentgenographic examination is adequate in itself if all the caries in a mouth is sought. If the findings are to be used as a basis of treatment, it is imperative that the greatest possible number of lesions be detected. This cannot be done even by the most meticulous clinical examination, such as was followed on the patients discussed in this report. Unless bite-wing films are used in diagnosis, approximately one-third of the patients possess undetected cavities; the use of full-mouth surveys, in addition to clinical and bite-wing examinations, increases the number to approximately 50 per cent. In addition, nearly twice the number of cavities per child are found by the use of the full-mouth survey. On the other hand, if the examination is used as a basis for evaluating the caries picture between groups of children used for experimental studies, the roentgenograph is of such little value in changing the score that it may be considered dispensable.

2. A comparison of groups of children exhibiting high and low total caries pictures was performed in an effort to determine what percentage of additional cavities were contributed by the roentgenograph. Although the less susceptible group demonstrated a greater ratio of enamel caries, about the same total relative increase was noted per child between groups using bite-wing films. A significantly greater number of lesions were found, however, in similarly carious groups using the full-mouth survey.

3. No outstanding sex differences were manifest in caries incidence or relative number of cavities found by roentgenograph in any of the comparisons.

4. The number of times a child had been previously examined or the age had no apparent influence upon the number of cavities found by the roentgenograph following the clinical examination.

Acknowledgment is gratefully made to Dr. Julian D. Boyd of the Department of Pediatrics, College of Medicine, who offered the suggestion that a study be made of the roentgenographic material on groups of children, and who is responsible for the research project in operation at Glenwood. The roentgenographic illustrations were furnished by Dr. Duane Lovett of the College of Dentistry.

REFERENCES

1. Day, C. D. M., and Sedwick, H. J.: Studies on the Incidence of Dental Caries, *Dental Cosmos* **77**: 442, 1935.
2. Delabarre, F. A.: Preschool Age Dentistry, *J. A. D. A.* **20**: 124, 1933.
3. Fixott, H. C.: Use of Roentgen Rays in the Practice of Children's Dentistry, *J. A. D. A.* **24**: 91, 1937.
4. White, D. P.: Preliminary Bite-Wing Roentgenographic Examination, Naval Aviation Cadets, *Naval Med. Bul.* **43**: 901, 1944.
5. Smith, P. K.: The X-ray—An Essential to Diagnosis and Prognosis of the Child Patient, *J. A. D. A.* **29**: 796, 1942.
6. Bodmer, E.: Diagnosis of Dental Caries by Means of Oral (or Clinical) and Radiographic Examination, *Northwest. Univ. Bul.* **39**: 2, 1939.
7. Sognnaes, R. F.: The Importance of a Detailed Clinical Examination of Carious Lesions, *J. D. Res.* **19**: 11, 1940.
8. Boyd, J. D., and Cheyne, V. D.: Epidemiologic Studies in Dental Caries. 1. The Pattern of Dental Caries in Iowa City Children, *J. Pediat.* **29**: 157, 1946.
9. Barr, J. H.: The Diagnostic Value of Radiographic Examination for Proximal Caries in the Deciduous Posterior Teeth, *New Zealand D. J.* **41**: 89, 1945.
10. Cohen, M. M.: Clinical Studies of Dental Caries Susceptibility in Young Diabetics, *J. A. D. A.* **34**: 239, 1947.
11. Boyd, J. D., and Drain, C. L.: The Arrest of Dental Caries in Childhood, *J. A. M. A.* **90**: 1867, 1928.
12. McCall, J. O., and Wald, S. S.: Clinical Dental Roentgenology, Philadelphia, 1940, W. B. Saunders Company, p. 172.
13. Arnett, J. H., and Ennis, L. M.: Dental Infection and Systemic Disease, *Am. J. M. Sc.* **185**: 777, 1933.
14. Burket, L. W.: The Accuracy of Clinical and Roentgenologic Diagnosis of Dental Caries as Determined by Microscopic Studies, *J. D. Res.* **20**: 71, 1941.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the New York Section

School of Dental and Oral Surgery, Columbia University, October 30, 1947*

Compiled by SAMUEL C. MILLER, *Editor of New York Section*

1. Development and Morphology of Physiological Secondary Dentin. *Sidney Benzer,† College of Dentistry, New York University.* The examination of ground sections of one hundred permanent normal erupted and forty unerupted teeth, multi- and single-rooted, of various age levels, discloses a layer of dentin which is formed after primary dentin is deposited. There is usually a sharp line of demarcation between the original "primary" and the new layer called "physiological secondary dentin." The chronological development of this tissue was traced in specimens ranging from seven to sixty-five years of age. Detailed study reveals several morphological types of physiological secondary dentin. The physiological secondary dentin appears in normal, permanent, deciduous, unerupted, and ovarian cyst teeth, as do the other dental tissues which include enamel, primary dentin, cementum, and pulp. This indicates that this tissue is a normal component of dentin and is not the result of function, irritation of oral fluids, or caries, as previously reported.

2. Oral Manifestations in Rats Fed Synthetic Diets Deficient in Pantothenic Acid and Biotin. *Daniel E. Ziskin, Maxwell Karshan, and David A. Dragiff, School of Dental and Oral Surgery, Columbia University.* This was a study of the changes in the oral tissues of rats fed a series of synthetic diets complete in every respect, except pantothenic acid, panthenol (a synthetic pantothenic acid), biotin, and folic acid which were the experimental variables. In diet No. 1, the four vitamins were omitted for some animals, added singly for others, and given in combinations in a third group. In diet No. 2, egg white replaced the casein. Similar variations of pantothenic acid, panthenol, biotin, and folic acid were used. Two hundred twelve weanling rats were divided equally as to sex. By the end of the twelfth experimental week, the rats either died or were sacrificed. The skin manifestations, blood chemistry, blood counts, and weights were noted. Those animals receiving diet No. 1 unsupplemented by the experimental variables were chiefly on a pantothenic acid-deficient diet, while diet No. 2, unsupplemented, emphasized a lack of biotin. Unprotected groups subsisting on diet No. 1 showed ulcers of the tongue in various sizes characterized by the granular type of necrosis described in previous reports. Unprotected groups subsisting on diet No. 2 showed, grossly, clear and hemorrhagic vesicles on the tongue of subepithelial or subdermal origin. The histological phase of this study is incomplete. An evaluation of the protective properties of pantothenic acid, biotin, and folic acid was based on the oral and skin symptoms. The addition of pantothenic acid to diet No. 1 seemed to afford the best protection in the class of single supplements. One addition of biotin to this diet gave some alleviation, while folic acid was ineffective. Combining pantothenic acid and biotin gave almost complete protection. In diet No. 2, biotin alone did not completely protect against the development of symptoms. The addition of pantothenic acid was found to be of little value, while folic acid aggravated the symptoms. The action of panthenol was comparable to that of pantothenic acid under all conditions.

Received for publication Nov. 28, 1947.

*Presiding, Frances Krasnow, Chairman.

†Guest.

3. Radioactive Phosphate Permeability in Dentin Following the Use of Medicaments. *Melvin Amler,* College of Dentistry, New York University.* Various medicaments commonly used in dental practice have been reported to affect the permeability of dentin. Previous studies have shown that they frequently affect not only the permeability, but cause reactions in the pulp tissue. In this investigation various medicaments commonly used in dental practice were tested to ascertain their effect on the permeability of the coronal dentin of normal dogs. Cavity preparations were treated with different medicaments, and then radioactive phosphate (in solution as Na_2HPO_4) was sealed in the respective cavities with silver amalgam. Controls were also prepared in which the cavity preparations were not treated with any medicaments prior to sealing in P_{32}. The teeth were later sectioned (ground) and placed on ultra speed x-ray film; the resulting radioautographs showed the following: The oxyphosphate cement base was completely impervious to P_{32}. Penetration of the dentin was highest in the phenol-treated cavities, less, in the order indicated, when phenol was followed by alcohol, fluorine, silver nitrate reduced with eugenol and cavity varnish. It is significant to note that in all cases, except with oxyphosphate cement, the action of the various medicaments apparently increased the permeability of the dentin as compared with the control preparations in which no medicaments were used.

4. Epithelial Changes of the Tongue in Rats Fed a Protein-Deficient Diet. *Daniel E. Ziskin and George Stein, School of Dental and Oral Surgery, Columbia University.* Ten immature albino rats (45 to 60 grams), five males and five females, were fed a synthetic diet complete in all respects except for the omission of casein. This experiment was in the nature of a pilot study for a more elaborate investigation of the influence of protein-deficient diets on the oral tissues. The animals refused to eat adequate amounts of the diet offered, but their appearance differed from that seen in inanition. They lost considerable hair, and that which remained arranged itself in pointed tufts giving the animal a porcupine-like appearance. They were irritable and developed severe neurological symptoms which affected their gait. Terminally, some showed swellings on the face or torso which at autopsy proved to be amorphous, colorless, gelatinous masses. Their tongues appeared smooth and were bright red in color. Weight loss was progressive, the final weights being equal to those at weaning (35 to 40 grams). Histological examination of the tongues disclosed an alteration of the epithelium never before observed by us under experimental conditions. It consisted of a reduction in the thickness of the epithelium of the dorsum with replacement of the dermal and cornified papillae by smooth, cornified epidermis so that the entire epithelial covering of the tongue resembled the epithelium normally found on the under surface. This change, in varying degrees, was a constant finding. Two cases receiving casein after marked clinical symptoms were manifest showed a reappearance of the papillae.

5. Electrophoretic Determination of Protein Fractions in Saliva. *Robert J. Gorlin,* School of Dental and Oral Surgery, Columbia University.*

*Guest.

PROCEEDINGS OF THE UNIVERSITY OF MICHIGAN SCHOOL OF PUBLIC HEALTH AND SCHOOL OF DENTISTRY INSERVICE TRAINING COURSE FOR THE EVALUATION OF DENTAL CARIES CONTROL TECHNICS

September 8 through 13, 1947

MONDAY, SEPTEMBER 8

INTRODUCTORY REMARKS

DR. KENNETH EASLICK

You people have been called together in this Auditorium of the School of Public Health to do a job that seems desirable and very much needed. How much needed you may expect to learn from the second morning speaker. The job is, as you know, the evaluation of the scientific information which is available on the process of dental caries and its control, as far as it is humanly possible as of September 8-13, 1947.

A job to be completed successfully calls for effort and rolled-up sleeves. Sometimes, as you may recall, it calls for blood, sweat, and tears; I hope that we do not go that far at this meeting. As I assess this week of planned activity, all of us are going to have to work hard and quite continuously for six days, but I think that all of us are going to like it.

Because of some pointed questions overheard, "What is an inservice training course, anyhow?" it seems particularly appropriate that the Planning Committee selected for the "kick-off" speaker this morning the man that it did, and I personally should like to congratulate Dr. Henry Vaughan on his attendance. The last time that I presided at an Inservice Training Program in this auditorium, Dr. Vaughan, at the very last minute, begged to be excused from speaking—he had an appointment with his dentist. Sometimes it is a real achievement to secure Dr. Henry F. Vaughan, Dean of the School of Public Health, and to find him willing to speak briefly on the subject which our Planning Committee assigned to him, "The Inservice Training Program at the School of Public Health." Dr. Vaughan, we appreciate the keeping of your appointment this time with 70, or 80, or 100 dentists instead of just one.

THE INSERVICE TRAINING PROGRAM OF THE SCHOOL OF PUBLIC HEALTH

HENRY F. VAUGHAN, DR. P. H., DEAN, SCHOOL OF PUBLIC HEALTH, UNIVERSITY OF MICHIGAN

I do appreciate this opportunity of "kicking-off" the ball, as it were, at this intriguing, enticing, and I am sure to be most profitable conference. It may be somewhat misleading to use the term "inservice training course" in connection with an occasion of this sort when the grandpappies, like myself, put on their spectacles to participate in the discussions, but when we started this School of Public Health a few years ago, we were quite intent upon having some mechanism for continued education. And many of our neighboring schools, notably the University of Minnesota, have done such a grand job in this respect that we felt that in so far as those phases of education are concerned which impinge upon the health field, we wished to encourage a periodic opportunity for those who are career people in public health, or who serve on the periphery of public health, either professionally or as lay individuals, so that they can

have an opportunity of coming together to discuss common problems and to lend their influence to the fulfillment of knowledge with regard to their particular field of professional or trade practice. So, since 1941, which is only a matter of six years, we have had these "inservice training courses," which were made possible in the beginning through the financial support of the W. K. Kellogg Foundation, for people who are in public health career work as a full-time avocation, for others who are in medicine, dentistry, and allied professions, which border upon public health, and we have had some very successful "inservice training courses" for those who are by public position, or otherwise, interested in matters of public health, such as people in the field of sanitation who are engaged in the operation of milk plants, water plants, sewage plants, hotels, restaurants, and people who are concerned with the manufacturing of articles and projects and gadgets which are to be used in the interest of public health.

You will be interested in some of the more recent "inservice training courses" that we have had. Just a year ago this week we had a conference here on "Local Health Units for the Nation," which is known to many of you, especially those of you who subscribe to, and read, occasionally, the *American Journal of Public Health*, because our proceedings were published in that journal in January as a supplement. That course was put on in cooperation with the conference of State and Territorial Health Officers, in cooperation with the American Public Health Association and the Schools of Public Health. For a week we focused attention upon completing the coverage of the United States with full-time local health service, a problem not unlike your own. Yours is one phase, one portion of that total problem of adequate public health service. You may be interested in knowing that this same group that met here a year ago under the auspices of the Committee on Local Health Units of the A.P.H.A. is convening this week at Princeton to discuss further the coverage of the United States with local health units, not this time with the state health officers, all of whom were here last year with their directors of local health service, but this time with the heads of the nonofficial health agencies of the United States. Organized dentistry and medicine along with the tuberculosis group, the cancer group, organized labor, and other groups will be represented at this Princeton conference. Again we shall be focusing attention upon the completion of the coverage of the United States with local health units. Some 40,000,000 people still reside in areas where there are no full-time local health units. Thus in each one of these "inservice training courses" we have tried to have a purpose at hand.

Later that same month, a year ago, we had here all of the professors of preventive medicine of each medical school in the United States, 77, and every medical school in Canada, and several from abroad, including representatives from England. We focused attention upon the better teaching of preventive medicine in medical schools, an important and a very difficult problem. When we convened here we called ourselves an "inservice training course." So, the term need not frighten you; it need not substract from the importance of the

occasion. And it seems to me that it is a very appropriate one, after all, because we are all being trained in some sort of service or other.

In the field of evaluation of practices we have had several conferences here under the auspices of the Committee on Administrative Practice of the American Public Health Association with representatives from most of the states in the central Mississippi Valley, and we shall have more occasions of that character as time goes on. The one thing which limits our courses is the housing situation in Ann Arbor. We hope that this situation will improve, and that we may have more than five, or six, or seven of these courses annually.

Now, to turn our thoughts for the moment to this important occasion. As a public health officer of many years of experience, and to those of you who do not know me, I may say that I served in a large city as a city health commissioner for twenty-five years. I have always been impressed with the lack of objectivity so far as our dental program is concerned. The dentists themselves have gone all out for the improvement of oral health in some of our cities. Thirty years ago, under the aid of the late Dr. Charles Oakman, the dentists of Detroit circularized through their own offices a petition which created so much public interest in dental health that the City Council in one year appropriated $100,000 to the Health Department to start a dental health program—thirty years ago. And what was done? The usual program of examination, inspection, and the filling of cavities and the routine, around the same circle, went on for years without a truly objective program based on the underlying factors in a dental health program. And health officers, I can tell you, are hungry for word from you, for suggestions, as to how a dental program should be established on a firm basis with a 1, 2, 3, or 4 statement of what the program should consist, one which will be scientifically designed and practical in its application. And it is with this objective in view that Dr. Easlick and the Planning Committee have asked you to convene here in Ann Arbor for this week, with the hope that your expressions will not only be self-satisfying, but that they will have a material influence upon the work of health agencies throughout the nation. What you do here will not be provincial in character; it will receive expression throughout this country, and in fact, in other countries as well. So, I think, Dr. Easlick, that this is a most important occasion, one of the most important ones that we have had. Out of it will come some recommendations which will either leave us hopelessly befuddled, as we are at present, or which will result in a more scientific, effective, and more economical utilization of the funds which are available, and which surely must become available to improve dental health among our people.

We are delighted that you are with us here in Ann Arbor. I hope that it is not going to be too warm. I think it will probably cool off within a day or so—at least that is my fond expectation. You may be needing your overcoats before the week is over; you can never tell what is going to happen in Ann Arbor. The School of Public Health, as well as the School of Dentistry, are to be your homes while you are here, I hope that you will make full utilization of this opportunity.

Thank you very much, Dr. Easlick.

DISCUSSION

DR. EASLICK.—Thanks so much, Dr. Vaughan.

I hope that everyone now knows "what is an inservice training course."

To get you prepared for the next speaker, we have distributed some mimeographed material, entitled "The Critical Appraisal of Dental Caries Research—Its Importance to Progress in Oral Health."

As I stated earlier, the need for this workshop job is to be elaborated very pointedly by our next morning speaker. Before I turn this task over to him, I should like to make two observations: (1) I am amazed often how neatly and quickly a big health fad, myth, or unscientific observation, conclusion, reaction, opinion or concept is dispatched by a small bit of well-planned research; and (2) I find it much more amazing how long, after the neat, quick demise of an untruth, does it linger around unburied in full vigor, vitality, and virility with the dental profession, with the allied health professions, with schoolteachers, and with the public.

Now, these two observations, I think, supply the basis for the background of groans and gripes with which two Councils of the American Dental Association hold their meetings. These observations are, I know, the reason for the presence of a W. K. Kellogg Foundation Institute of Postgraduate Dentistry at this University. "Basic research," it has been said, "constitutes the lifeblood of a health profession," and I think most of us are willing to agree. But many of us also will agree that, of equal importance, is the rapid dissemination of research findings. The continual dissemination of new information, in fact, is the prime reason for postgraduate education, certainly in the health sciences, and it should, it would seem to me, constitute one of the prime objectives of all health education—certainly of dental health education.

The two Councils of the American Dental Association which are so concerned with what is fact in dental health are the Council on Dental Therapeutics, which promotes the scientific use of rational drugs, as you know, and the Council on Dental Health, which promotes scientific health statement by the profession and the public. And don't, for one moment, fail to remember that the path of each has been a bit thorny, and still is full of thorns. I think because of some of this background both Councils helped to promote this program.

Now, so that you, who are assembled here from all over the English-speaking portion of the North American Continent—and I should like to pay my respects to our Canadian delegation—so that you will not forget that there is a thorny path of fact, the Planning Committee has invited the Executive Secretary of one of these accrediting groups of the American Dental Association to follow Dr. Vaughan's "kick-off" here this morning with a "keynote" address. And it is a very nice pleasure for me, as a member of the Council on Dental Health, to present as the keynoter—and he states that it will be a bit difficult to follow his keynoting unless you follow it on his mimeographed outline (see next page).

THE CRITICAL APPRAISAL OF DENTAL CARIES RESEARCH— ITS IMPORTANCE TO PROGRESS IN ORAL HEALTH

By Donald A. Wallace

Introduction

I. General considerations.
  A. Means of caries control.
    1. Decrease of forces which tend to cause caries.
    2. Increase of tooth resistance.
  B. Mechanism of caries is not well understood, and therefore methods of prevention are largely empirical. Clinical proof crucial. Many control measures act through more than one mechanism.

II. Measures which have been proposed for control of caries.
  A. Systemic.
    1. Vaccines.
    2. Hormones.
    3. Nutritional agents.
      1′. Vitamins (A, C and D).
      2′. Minerals (Ca, P and F).
      3′. Limitation of sugars and starches.
  B. Mechanical.
    1. Operative dentistry and orthodontia.
    2. Toothbrushes, toothpicks, dental floss, and abrasive dentifrices.
    3. Therapeutically inert mouthwashes.
    4. Abrasive or detergent foods or confections.
  C. Chemical.
    1. Detergents (''luster-foam,'' Na ricinoleate).
    2. Impregnating and Surface-changing agents ($ZnCl_2$, $AgNO_3$ and Fluorides).
    3. Enzyme inhibitors (Menadione).
    4. Alkalies (Milk of Magnesia, $NaHCO_3$).
  D. Bacteriological.
    1. Antiseptics.
    2. Antibiotics.
    3. Ammonia derivatives and quinine.
    4. Implantation of bacteria.

Criteria for Appraisal of Caries Control Studies

I. Qualifications of the investigator.
  A. Training.
  B. Experience.
  C. Available experimental subjects.
  D. Available equipment, funds and personnel.

II. Selection of preventive or therapeutic measures.
  A. Safety.
  B. Simplicity.
  C. Acceptibility to the public.
  D. Availability to the public.
  E. Cost.
    1. In financial terms.
    2. In terms of professional time.
  F. Likelihood of abuse.

G. Composition and degree of purity of test materials.
    1. Complete quantitative composition of all foods and drugs used should be known and reported.
H. Blind test methods should be employed where practical.
I. Methods and preparations used should lend themselves to immediate practical application through established facilities.
J. Mechanism of action of drugs should be understood before general use is recommended.

III. Selection of test subjects.
A. Number.
B. Distribution by age, sex, strain, race, environment, diet, habits, health status.
C. Establishment of experimental and control groups.
D. Adequate discipline.
E. Stable population.

IV. Preliminary survey and observation of test subjects.
A. Diagnosis.
    1. If systemic effects are possible, this should include general physical examination with special attention to parts of the body most likely to be affected.
B. Observation of test subjects for an adequate period before preventive or therapeutic measures are started.

V. Duration of the study.
A. Carious lesions progress slowly in many instances, and sharply defined dates for initiation of the carious process cannot be set.
B. A post-experimental period of observation may be desirable.

VI. Statistical evaluation of results.
A. Multiplicity of inadequately controlled observations is futile.

Importance of Critical Appraisal to Oral Health

I. Methods known to be effective should be used until better methods are established by research.

II. Caries-prevention does not of itself lead to oral health.
A. Regular prophylaxis and other dental office treatment are necessary.

III. Reliance upon ineffective or hazardous methods is inadvisable.

IV. Any method for control of caries must be accepted by the profession and the public before it will be practically effective.

Dr. Donald A Wallace of the Council on Dental Therapeutics speaking on the subject assigned to him, "The Critical Appraisal of Dental Research—Its Importance to Progress in Oral Health."

THE CRITICAL APPRAISAL OF DENTAL CARIES RESEARCH—ITS IMPORTANCE TO PROGRESS IN ORAL HEALTH

DONALD A. WALLACE, PH.D., COUNCIL ON DENTAL THERAPEUTICS, AMERICAN DENTAL ASSOCIATION, CHICAGO, ILL.

Before going into the discussion of the criteria which may be used in evaluating research, let us consider the basic means of caries control. None of this is new; it simply reviews what other people have said. You can control dental caries by decreasing the forces which tend to cause the caries, or you can control it by increasing the resistance of the tooth to the attacking forces. I think all of you know that there are many examples of methods which fall into both categories. As one example, the limitation of carbohydrates in the diet may decrease the attacking forces, and the topical application of fluoride solution to the teeth may increase tooth resistance.

The mechanism of caries is not well understood. We should start out with that firmly in mind because we have all read papers which indicate that one or another idea of the caries process has been more or less finally proved. So far as I can determine none entirely has been so proved. Since the mechanism of the caries process is not understood, the methods of prevention have to be largely empirical, and therefore, a clinical test is the only way that we can determine whether the methods suggested are effective. Many of the control measures act through more than one mechanism, but I have attempted to classify them perhaps a little differently than they are classified in the outline for the inservice training program. I have them classified as systemic, mechanical, chemical, and bacteriological procedures. Each one of these will be discussed by other speakers and I shall not go into detail concerning them now.

The first criterion for appraisal of caries control studies, or any other research, it seems to me, is the qualifications of the investigator. In general, the private practitioner does not have the background or the facilities to conduct a caries control study. The best he can do is to cooperate with some individual or group which has that background and facilities in the assembling of information, which in the aggregate can be evaluated. The clinic patient can be used as an experimental subject much more properly, as a rule, than a private patient; therefore, the experimental subjects in many instances would be drawn from large groups.

The background of experience of the investigator is important, and it should, as a rule, include some formal training in research. The investigating group should have an adequate number of experimental subjects available. There again, that is not usually true of a private practice. They should have ample equipment, funds, and personnel because these caries control studies usually last at least two years. Such studies usually involve hundreds of subjects if the results are to be significant, and usually they should be supervised by full-time personnel.

The selection of preventive or therapeutic measures involves, first, the consideration of safety. Since caries is not a fatal disease, and people can live without their teeth, it is not justifiable to use drastic methods or drastic materials to control caries. In general, one must assume that any caries control method should be harmless. Second, the simplicity of the method is important. Third, it should be acceptable to the public. For example, I think that we could control caries in the United States very well if we could get people to control the amount of carbohydrates they consume, but that method is not acceptable to the general public and therefore, even though it may be the best method that is available today, it is not of a great deal of use to us as a public health measure.

Fourth, the procedure should be available to the public. Fluorination of the public water supplies will affect only perhaps half of the population because only about half of the people get their water from a general public supply. Any advice that is given out with regard to tooth brushing, oral hygiene, will only affect about a fourth of the population, or perhaps a little more at this time, because only about a fourth of the people brush their teeth regularly. Topical application of fluorine in the dental office would be available only to those who visit their dentist.

The cost must be figured both in financial terms, that is, the cost of the drugs or the apparatus required, and in terms of professional time. If it takes longer or costs more to prevent caries than it does to repair its initial ravages, the prevention method is not as good as it should be.

Then there is the likelihood of abuse. For example, today perhaps a good many physicians are treating patients with synthetic calcium fluoride tablets. So far as we know, those tablets have no value in caries prevention, and in many instances the people who are being treated by means of them undoubtedly are being lulled into a false sense of security which may cause them to postpone adequate dental care.

The composition and degree of purity of test materials are important factors. Many times you will read scientific papers in which it is stated that a certain chewing gum or tooth paste, or a certain type of food is used, and it is obvious that the author thinks that Blank's tooth paste is the same product today as it will be tomorrow, or as it was yesterday. We know from our experience with these products, in all fields, not just in tooth paste, that their composition is highly variable. Sugar will be a constituent of some tooth pastes when the price of glycerin is high. Propylene glycol will be used when sugar and glycerin are both unobtainable. Similar considerations apply to chewing gum, and to other agents which are used as adjuncts or as the principal factors in caries control studies. Therefore, the investigator should satisfy himself about the composition of what he is using—make sure that all of the test material that he is going to use for the study has the same composition, and although he may wish to identify it by a trade name in his report, he should also identify it by a quantitative analysis so that the same material can be used in a later study if necessary.

Blind test methods should be employed where practical. It is not always possible to use placebos that adequately disguise the test medicament from the people who are participating, but it is known that where placebos are used in tests of therapeutic agents as many as 20 per cent of apparent cures of whatever disease is being treated may be obtained with the placebos. That may not be true of caries, where subjective effects are probably much more remote than they are in the common cold or certain other diseases, but the blind test technique is the most foolproof where it can be used. For the same reason the nature of the test material should be withheld from the investigators where possible so that they will not be prejudiced or biased in any way.

Methods and preparations used should lend themselves to immediate practical application through established facilities. I have already pointed out how fluorination of the public water supply has only about a 50 per cent potential, and how the application of fluorine topically in the dentist's office has probably a smaller potential. Such limitations should be considered with relation to practical application through established facilities. If it is necessary to set up a new agency to apply the method, that will make it less easily available, other factors remaining equal.

The mechanism of action of drugs should be understood before general use is recommended. This is more important if the drug is to be given systemically than if it is to be applied topically. There are instances in the history of therapeutics in which drugs which have been thought to be harmless and to produce miraculous effects have been found, after years of practical application, to cause side effects which were more serious than the condition which was intended to be improved by the use of the drug. Dinitrophenol is now almost a classic example. Streptomycin is following a little bit of that pattern, I believe. Until the mechanism of action is understood, widespread systemic use should not be recommended.

The selection of test subjects is important. It is axiomatic that a sufficient number of test subjects should be used, and in most caries studies that is a large number, particularly in clinical investigations. Distribution by age, sex, strain, race, environment, diet, habits, health status should be even between the controls and each of the experimental groups. The establishment of experimental and control groups is essential since uncontrolled studies are much less significant usually than those which are controlled, and especially by control groups right within the study rather than by information gleaned from public health records or some other remote source.

Adequate discipline of the subjects of the experiment is important, and for that reason orphanages and military camps are often chosen. A stable population is important because of the long period of time over which the study must be carried.

A preliminary survey and observation of the test subjects should include a thorough dental diagnosis, and if systemic effects are possible, as they sometimes are, this should include a general physical examination with special attention to the parts of the body most likely to be affected. The use of roentgen-

ograms as a diagnostic aid is well established where it is economically feasible. Observation of test subjects for an adequate period before preventive or therapeutic measures are started enables the investigator to satisfy himself that his groups have been properly selected, or to make exchanges among the different groups, if necessary.

The duration of the study should be adequate. Carious lesions progress slowly in many instances, and sharply defined dates for initiation of the carious process cannot be set. That is well illustrated, I believe, by the impression that is held in some quarters that dental caries is less common in sunny climates and in the summertime than it is in cloudy climates or in the wintertime. Obviously, since you cannot tell when the carious process starts, it is extremely difficult to say whether a person in Ann Arbor is developing caries in the summertime or the wintertime. The incubation period, you might call it, or the induction period, may be quite long. A postexperimental period of observation may be desirable. I think the desirability of that is obvious.

The results should be statistically evaluated, and of course in the use of statistics, as in the use of any other mathematics, the qualifications of the person who applies them are of paramount importance. Multiplicity of inadequately controlled observations is meaningless. In looking over research reports one should always compare the conclusions with the tables and other data given in the paper. Sometimes you will find that the conclusions do not adequately represent the data in the paper. There may be data which the author does not think are important, but which turn out to be highly significant.

Methods known to be effective should be used until better methods are established by research. Techniques of operative dentistry should not be slighted, and the recommendation that the proportion of the fermentable carbohydrates in the diet be kept at a minimum should still be made until we have other methods that can replace these.

Caries-prevention does not of itself lead to oral health. Even if we find a completely effective means for preventing caries, the dentist will still be needed.

Any method for control of caries must be accepted by the profession and the public before it will be practically effective.

Thank you very much.

DR. EASLICK.—Thanks much, Dr. Wallace, for your discussion, and for working out this mimeographed statement. We liked your "keynoting."

Since we are planning on considerable audience participation, I think that we should pause right now perhaps and ask for discussion—ask for questions. We certainly have a period here in which we can take questions from the floor, or we can take discussion. So, if anyone here—or let me put it this way—if Dr. Wallace has trodden on anyone's tender bunions, blisters, festers, or fetishes, I shall throw the meeting open for a period to discuss them, or to question, or to arguments, so that Dr. Vaughan can't escape—

DR. VAUGHAN.—I don't want to escape.

DR. EASLICK.—If there are any questions or discussions to be raised about the School of Public Health here, the setting and background, or an "inservice training program," we will accept questions directed at him. Do I hear any from this audience?

(Hearing none)—I think there will be some later.

DR. HENRY C. GAGE.—(Colorado Dental Health Service.) This word "placebo" medication I am not clear on that.

DR. WALLACE.—A "placebo" is a medication which is therapeutically inactive, which resembles the active medication. For instance, sugar pills are often used as placebos.

DR. LEONARD S. FOSDICK.—(Northwestern University.) I notice Dr. Wallace states here, "The mechanism of caries is not well understood, and therefore methods of prevention are largely empirical." I think that he is quite right in stating "largely empirical," but I think it should be brought out that practically all of the previous really important research problems have started out on a basic theory that is quite well understood, and then experiments are set up on a logical basis, rather than empirical, on the basis of the theory. Then, of course, if the theory is proved—or if the experiment proves well that strengthens the theory, but if it is not so good that weakens the theory. So, I think it should be stated here that although it is not completely understood there are several basic conceptions of which there is agreement.

DR. WALLACE.—I want to thank Dr. Fosdick for making that clear. I did not mean to disparage the theoretical approach to research in any manner, I was just simply pointing out the condition which exists in caries research.

DR. EASLICK.—If nobody wishes to pursue the discussion further we shall proceed with the program. The next speaker, I am sure, is very excellently described by that hackneyed phrase, "he needs no introduction." At any rate, he is not going to get an introduction because nobody has been retained to do the honors. The Planning Committee decided that I should take the next spot on this program and state, as clearly as possible, some of the program's background, its objectives, and the organization by which it is hoped to achieve those objectives. So, it is with hesitant pleasure that I introduce me to you.

OBJECTIVES AND ORGANIZATION OF THE CARIES CONTROL WORKSHOP

KENNETH A. EASLICK, A.M., D.D.S., PROFESSOR OF PUBLIC HEALTH DENTISTRY, UNIVERSITY OF MICHIGAN, ANN ARBOR, MICH.

An alumnus of the University of Michigan* (and we here in Ann Arbor always are partial to the statements of graduates of the University of Michigan) stated recently that, to a considerable degree, the history of man is the history of myths. Myths, probably to most of you, suggest the folkways of primitive peoples and of ancient civilizations. I think that you will find, however (you will if you join the members of the Council on Dental Health in their evaluation of the dental health statements in school textbooks!), that many ancient myths still are believed and some new ones actually are being made. Some of these new ones appear to us on the Council on Dental Health as more detrimental to human welfare than the old ancient myths that are a half or a quarter believed.

And I should like to pursue this thought just a moment further. Myths flourish by repetition and the tradition of authority. I can point out a statement in one dental textbook which quoted an "authority" in its first edition and repeated him for five successive editions. The statement of the authority had been killed abruptly by a piece of laboratory research some years before the printing of the original textbook, the very first edition.

I told an unappreciative representative of a book-publishing company recently, when he annoyed me about writing a book, that textbooks make excellent mausoleums for burying dead knowledge and that I hoped the purchasers merely bought textbooks to fill up empty shelves and that it was a shame to disturb dead things because so many of them smell vilely. And I think that many of you may have had the same discouraging experience as have I; you have scanned a new text and found statements that were already out of date by the time it was published. Now, right here I think that I should pause, perhaps, and offer my apologies to any of you brave ladies and any of you courageous men in this auditorium who ever have written a textbook.

In passing, it may be worth while to note that myths also flourish by the coercive control of learned societies and distinguished groups. Then myths die hard, because it is difficult for scientific fact and common sense to demonstrate the myths' absurdity and harmfulness. They seldom die a real decent death when pompous individuals will to "*believe*" rather than to "*conclude.*"

Any scientific hypothesis should survive when verified by fact and any unscientific hypothesis should die a clean-cut and final death when found invalid. It is hard to envision a true scientist who would try to preserve a poorly conceived hypothesis by burning people at a stake, putting them in concentration camps or even branding them as un-American. The true scientist forgets his "preliminary reports" and his once intriguing hypothesis and goes to work on

*Bain, Read. Man, the Myth-Maker. *Scientific Mo.*, 65: 61-9, July, 1947.

something new and more productive. And, Ladies and Gentlemen, it is something of this point of view which I have hoped can be interjected into this week's work.

Time after time, when our dental students are assigned a critical appraisal of information, they come back to one of us on the staff with the complaint, "But, Doctor, how am I going to tell who is an authority?" Usually they are referred back to the instructions in a small manual* which has been supplied to them. I said "manual" not textbook. And I will repeat some of the statement in this manual to you this morning. I don't think it will be quite competitive to the statements by Dr. Wallace, but maybe it will bolster some of them—at least I hope.

This manual says,

"Possibly the first questions to be asked, in the evaluation of information, deal with the *publication* in which the article is printed. One asks, is the journal published by a critical, scientific, professional society? Is it the publication of an uncritical trade organization? The material in some highly scientific journals is submitted to the rigors of a board of censors; while some journals are compelled to accept any material that is available in order to put out a publication.

"The second question concerns the *writer* of the article. Has his record in the past demonstrated that he has a careful, accurate, scientific attitude?

"Then one asks about the *setting* in which the writer is working. Does he belong to the staff of a hospital, a foundation, a research clinic or a university, or does he write from the background of isolated private practice? Is he working with a group that has available ample equipment and clinical library and consultative services, or is he working with a mediocre group that is inclined toward hasty conclusions and frequent reporting of them?"

Then continues the manual—

"Study of the *statement* of the writer provides the final criterion of evaluation. The introduction of the article, alone, may indicate that it reports painstaking research, careful observations or clinical investigations. If the writer wishes to have others accept his statement as truth, he must substantiate his conclusions by acceptable scientific data. His conclusions must be stated with an open mind at all times and be made critically without prejudice. It seems impossible to seek truth once one has acquired a biased opinion.

"If the writer is reporting research investigations, one may ask, has he set up his laboratory or clinical situation to exclude modifying factors and has he provided satisfactory controls? Any article reporting conclusions based on experimental evidence should submit enough data in order that the reader may be able to check results. Usually, one may expect summarizing charts or tables and at least enough of the mathematical procedure employed to enable the reader to determine how the conclusions were derived and to permit the application of statistical tests, if one wishes, to check the validity of these conclusions."

*Report writing in dentistry (Ann Arbor, University of Michigan, 1946.) 26 p. processed.

Then our little manual continues—

"The subjects utilized for the research should be described accurately as well as the conditions under which the experiment was performed or the observations made. No essential portion of the experiment should be left to the reader's imagination. Unless this essential information is supplied, faulty technics may go undetected.

"The reader must not be impressed unduly by 'large sample' experiments. A large group of subjects with mixed characteristics may supply information of less value than that from a small group of subjects more carefully selected. It always is well to question, is the sample significant? On the other hand, it is not fair to ignore data obtained from well-planned studies utilizing small samples, since these studies can supply very reliable information. It is frequently of value to ask, could other conclusions be drawn with equal validity from the data submitted, or could it be that chance, alone, is operating? Human beings are prone to credit a cause-and-effect relationship to coincidental findings—'post hoc, ergo propter hoc,' said the Romans!" And perhaps the Romans had a thought.

To get back to this Caries Control Training Program, something of this attitude, too, I have hoped might be interjected into this week's activities, and I was pleased when Dr. Wallace brought along his outline for the evaluation of information. At least we should like to tackle our job with a productive attitude. That is why Dr. Marguerite Hall and her entire staff of vital statisticians will serve you people as consultants during this period. They will be added to some of the dentists in this group, who I know have a very excellent background in statistical treatment. That is why, too, Miss Hilda Rankin and an augmented staff of librarians are available in the School of Dentistry to supply you promptly with the documented evidence submitted by our imported faculty during the week, and any other evidence which you wish to discover on your own initiatives.

Now, in order to present some further details that may be helpful to you during the remainder of the week, I shall have to report some of our early planning. Sometime last January a letter went out to each member of the advisory group to settle the theme for this week of study. Members of the group were given a choice of two themes, (1) the assembly of complete dental health data for planning an adequate children's dental program and its analysis at the state level and (2) the evaluation of "what-is-fact" in oral health information.

For a study of the complete problem that dentists would meet if they were to set out seriously to provide adequate dental health care for all of the children of a designated state, it was suggested to this Planning Committee that seven areas of information, of data or facts, would have to be gathered and explored. The first one was the adoption, the setting-up of standards of oral health care for children. These standards, the operations that we are going to perform for children that are adequate for their health, would have to be defined carefully at the beginning. The second area was to ascertain the population facts that were required. The third area of that experience would be the study of the

economic status of the designated state. And the fourth area to be explored would be to estimate by age groups the defined oral health needs of children either on the basis of their accumulated needs or on the basis of their incremental yearly needs. And the fifth area was a very complete census of all of the personnel of a state, of all who contribute to a children's dental program. And the sixth area was to prepare an estimate of the productivity of the state's dental personnel. And then, finally, the seventh area that we planned to explore, or suggested that might be explored, was the blocks to dental care. The blocks to dental care may be psychologic, economic, and even professional. Following this fact-finding excursion and accumulation of data, it was planned that a number of the pertinent problems in the field of dental economics would be proposed and then would be solved. That was one choice submitted for a Workshop.

Then, for the other choice, the "What-is-fact" Program, it was postulated, logically should receive priority since any planning to provide a complete dental health program for the children of a state should be preceded by the orderly evaluation of scientific information. Such an order of procedure would appear pertinent, at least, before clinical services finally are decided or dental health education programs launched to remove these psychologic blocks to dental care, that I just mentioned, or to educate people in preventive technics. It was stated further that four fields of "what-is-fact" in dental health should be evaluated thoroughly: (1) dental caries control, (2) maintenance of the health of gingival and supporting tissues, (3) occlusion and the interception of malocclusion, and (4) oral and systemic interrelationships. The vote of the planning group was registered almost unanimously for the "What-is-fact" conference and the first choice of areas of evaluation went to "caries control." As you may have guessed, should you react enthusiastically to the present discussion, we still have three other areas of dental health information to evaluate at later dates, then we shall be ready probably to start all over and return to dental caries control.

The planning for this week then proceeded as it was outlined in the preliminary letter. At that time it was decided that the inservice training course technic would be employed to appraise critically the present status of the various control measures now being made available by research and clinical observation. Lectures would be presented during the morning periods by sixteen well-known research workers in the fields of caries control, oral bacteriology, oral histopathology, biochemistry, nutrition, and periodontology and the afternoon sessions would be devoted to small group discussions and evaluations of six areas of caries control. As Dr. Wallace stated, we did not quite correlate with his very logical classification of areas of discussion. If you will consult the programs you will note that there are six committees to evaluate (1) the mechanism of the caries process; (2) relationship of systemic conditions to the caries process; (3) the effectiveness of prophylaxis, toothbrushing, chewing gum, dentifrices and mouthwashes, and the things which can be done at home by way of mouth hygiene in the control of caries; (4) the utilization of fluorides and silver salts

in the control of caries; (5) the relationship of vitamin and mineral ingestion to dental caries; and (6) the contribution of different types of diet to caries control.

Each evaluating group was to work under a designated leader with the assistance of research, statistical and library consultants, and also with the assistance of duplicated bibliographies of references which have been cited by the speakers. Also, along with these lists of references, so that you can subject the documented statements to the acid test, will be a list of questions, which it seemed to a group of us, should be raised and should be answered before a set of critical conclusions were written for presentation in the final session Saturday, September 13. That much already has been done by the planning group to start off the Evaluating Committees on Tuesday afternoon.

The activity of the six evaluating groups will determine the outcome of this conference—whether it is a success, whether it is mediocre, or whether it is just a plain failure. The planning group hoped that such an organized program would result, by the end of the week, in a brief statement of conclusions about (1) the factors that are essential for the caries process and (2) the scientific value of each control measure available. It was hoped, further, that these conclusions would be reached by a courteous, unbiased but extremely critical discussion of the validity of the data submitted.

In order to keep discussion on an objective, unemotional plane, the entire statistics division of the School of Public Health was drafted to reinforce the statistical training of some of our dentists, the talent that we already have in this group, so that you could turn to these technical people any time that you wished, have them apply certain statistical tests, and tell you whether the results were valid. It was hoped, finally, that the participants would shed all of their traditional respect for authority, coldly dissect research conclusions for their accuracy and unceremoniously state that more or better research is necessary when a question cannot be decided by the merits of existing data. Furthermore, as I have said, all of us hoped that you would enjoy the experience or we wouldn't have worked so hard to provide it for you.

Some features have been added unknown to the planning group, for example, there is to be a subscription dinner.

The faculty and group leaders already have held one session in which they were "briefed" Sunday evening. The lectures by our imported faculty all will be given in this auditorium. All of the activities of the evaluating groups are scheduled in the School of Dentistry and Kellogg Institute where seminar rooms will be available, and I hope cool, and where a well-stocked and well-appointed dental library is available, housing the data which, I think, the essayists at this meeting will submit.

That appears to be, perhaps a bit sketchily, your week-long program. I hope most sincerely, speaking as a very, very recent grandparent, that the results of this program will demonstrate consistently that a dental fact must become sufficiently grown up and strong enough, lustily to stand on its own two feet before it starts out in the world for acceptance as the basis for dental practice or for the education of the public.

Mr. Charles F. Kettering of General Motors' Research fame, whose pithy sayings I usually read and usually enjoy, once stated, "Before embarking on a new experiment it is well to have a rather definite notion about where you are going; otherwise you never will know when you get there." Adopting Mr. Kettering's thesis as a principle of action, I have tried this morning to draw a picture for you of where you are going this week. Such an effort appeared, to me, to be just common courtesy. It would seem that if one does not like what is about to hit him, it would be more comfortable to have an opportunity to withdraw early.

(Meeting adjourned for luncheon)

Monday, September 8

Afternoon Session

DR. EASLICK.—This morning we attempted rather seriously, in many ways, to provide you with an orientation experience to the week's program. Now that you have completed this freshman era successfully and purchased your notebooks, you are expected to tackle, with a truly scientific approach, some of the problems involved in the control of dental caries.

The first group of lectures to be presented in this auditorium in the pursuit of fact deals with the process of caries, the mechanism of caries. It seemed sensible to the Planning Committee to review that which is known scientifically about the process of caries before an evaluation of control technics is attempted. Possibly, the Committee surmised, some of the conflict of opinion and many misconceptions and a few dead-end paths could be avoided if everyone were exposed to a clear-cut concept of the factors essential for caries and the avenues, therefore, for interference with each one of these factors.

Probably, the Committee continued in its reasoning, the initial step in such a review of the process of caries should be a statement of the information to be derived from the embryology, the histology, and the physiology of the hard tissues most directly concerned in the caries process, i.e., the enamel and dentin of human teeth. Our neighboring State of Ohio, with which the pioneers of the State of Michigan, back in 1835, fought a bloodless war and lost Toledo, supplies the first afternoon speaker. This speaker undoubtedly worked the hardest of any of our imported faculty last night at the briefing meeting; so I know that he is well prepared today.

Dr. Samuel W. Chase, who is Professor of Histology and Embryology in the School of Dentistry of Western Reserve University, Cleveland, accepted as his forty-five-minute assignment "The Development, Histology and Physiology of Enamel and Dentin—Their Significance to the Caries Process."

THE DEVELOPMENT, HISTOLOGY, AND PHYSIOLOGY OF ENAMEL AND DENTIN—THEIR SIGNIFICANCE TO THE CARIES PROCESS

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My remarks are going to be concerned chiefly with those structural features of the dentin and enamel which seem to me to be most pertinent to the mechanism of caries. Since the enamel is the first line of defense against caries, it will be given more attention than the dentin. I do not need to go into an extensive account of the development of these tissues, yet I consider it necessary to say a few words about their development in order that we may, perhaps, better understand their adult structure.

The dentin begins to form at the surface of the dental papilla after the formation of a superficial layer of cells, the odontoblasts, which are close relatives of osteoblasts, or bone-forming cells. The dentin forms in much the same fashion as bone, a fact which was emphasized many years ago by von Korff,[27] whose name has been given to both the osteogenic and the dentinogenic fibers. The undifferentiated odontoblasts are closely comparable with, and perhaps derived from, the ordinary fibroblasts found in the dental papilla and later in the dental pulp. When dentin begins to form they change their shape, their cytoplasm undergoes alterations, and these cells, probably solely fibroblastic at first, become certainly more closely related to the process of dentinogenesis that some recent claims indicate.

For example, Gottlieb[21] has stated that the only function of the odontoblasts is to keep the dentinal tubules open. This claim, in my opinion, is not justified, since there is good evidence of active participation in dentinogenesis. Bevelander and Johnson[2] have shown that the odontoblasts become loaded with alkaline phosphatase just prior to and during early calcification of the dentin which indicates that they are in some way concerned with the precipitation of mineral salts. Also we have reason to believe that they are intimately associated with the formation of the fibrillar portion of the dentinal matrix. Orban[34] demonstrated excellently the relation of the collagenous fibrils of predentin to the Korff's fibers and other precollagenous fibers of the pulp, and Sicher[36] has recently presented convincing arguments associating the odontoblasts with the production of Korff's fibers. Sicher's paper is a sane, succinct and sound account.

As each layer of predentin is calcified the dentinal matrix is built up layer by layer, each odontoblast contributing a much branched, constantly elongating odontoblastic process lying in a similarly branched dentinal tubule. The cell-body of the odontoblast remains outside the dentin on the surface of the dental pulp.

Bodecker[5, 7] has long insisted on a hollow, tubular odontoblastic process by way of which the tissue fluid (his "dental lymph") flows into the dentin, to return by way of the space between the odontoblastic process and the wall of the dentinal tubule. Such a concept diverges widely from generally accepted beliefs regarding the nature of cytoplasmic processes and their relation to their environmental tissue fluid. The circular and U-shaped structures within the dentinal tubules in Bodecker's[5] illustration of sheep's dentin represent complete and partial turns of helices made by coiled odontoblastic processes much shrunken by pyridin used in preparation of the specimen, as can be demonstrated by measurements in similarly prepared sections. Other later illustrations purporting to show odontoblastic "tubes" can be imitated by causing precipitation of dyes on the surface of odontoblastic processes. The presence of vacuoles can also give the appearance of hollowness, but the odontoblastic process can safely be regarded as a solid cytoplasmic extension of the odontoblast.

Interglobular dentin appears in sections as areas bounded by segments of spherical surfaces of normal dentin. Such areas contain deficiently calcified dentinal matrix. Similar, much smaller areas are found in the granular layer in the root. Both have been described[1] as regions which favor the spread of dental caries when it penetrates into the dentin.

The nature of Neumann's sheaths has been the subject of much controversy. The fact that they are prominent parts of the walls of some dentinal tubules and not to be found in others is best explained by the view[34] that they represent dentinal matrix which remains in the predentinal condition.

The formation of dental enamel is among the most controversial subjects in dental histology and embryology. The exact mechanism is still a matter of opinion. Involved in the process are the tall ameloblasts, at least eight times as tall as they are wide. Near their basal ends they are invested by a prominent terminal bar appartus. Extending beyond this is a mass of cytoplasm, the ameloblastic (Tomes') process. The assertion, by Saunders, Nuckolls, and Frisbie,[35] that the terminal bars are artefacts caused by decalcification is certainly not correct. Molar tooth-germs from the same 3-day rat prepared with decalcification and without decalcification by the method used by these authors both show terminal bars, and Wassermann[39] has identified them in fresh enamel organs, before fixation.

When enamel is about to be formed there appear, within the cytoplasm of the ameloblasts, large numbers of somewhat basophil globules. These, in some unknown way, pass out from the cell, perhaps through the intermediation of the ameloblastic process, though they are rarely found in the process. The first substance to appear in developing enamel is a material which forms opposite the terminal bars in the position of the interprismatic substance. The prismatic substance is formed probably through the agency of, at least in the territory of, the ameloblastic processses. Sections through the earliest formed enamel show the typical "honeycomb" formed by the interprismatic substance, the "cells" of the "honeycomb" being occupied by the ameloblastic processes.

The enamel prisms are built up layer by layer, perhaps globule by globule, each representing a day's activity of the ameloblasts. The first enamel is relatively soft[41] but goes through a period of maturation. This was shown long ago by von Ebner,[17] whose findings have been largely confirmed by Kitchin[26] and myself.[11, 12] Following von Ebner's terminology the first enamel formed is called primary enamel. This markedly basophil substance changes into the acidophil young enamel. Later it becomes basophil again in the transitional phase of maturation. All three phases, primary, young and transitional enamel, are relatively insoluble in weak acids,[12] hence such immature enamel can be retained in sections.

The transitional phase accounts for a considerable amount of the thickness of the maturing enamel. Late in this phase resistance to decalcification is gradually lost, the enamel changing into von Ebner's phase of hard enamel, which is readily soluble in weak acids. Weinmann, Wessinger, and Reed[41] have shown and Deakins[14] has confirmed that the premature enamel has only 30 per cent of the mineral salts found in the fully calcified enamel. According to them the process of calcification involves removal of organic matter and water and the influx of mineral salts. Kitchin[26] has demonstrated a definitive crystallization at the end of maturation. Wassermann[39] confirmed my observation[13] that ameloblasts are essential to the progress of maturation and suggested that these cells are responsible for absorbing protein and water. Concerning the source of the mineral salts, various experiments with radioactive phosphorus[4, 23, 24, 31, 40] and recent observations by Wassermann[39] indicate that they come from the dental pulp by way of the dentin.

Gottlieb[19] has questioned the commonly accepted belief that each prism is formed by an individual ameloblast. According to him the early enamel is a uniform mass, the individuality of the prisms appearing in a later phase. However, since the relation of ameloblast to prism is constant and since, with the use of appropriate staining methods, individual prisms can be identified throughout all phases of maturation, there can be no doubt that each prism is formed throughout by one ameloblast.

Also, contrary to Gottlieb's[19] recent statements, it can be safely assumed that each enamel prism extends from the dentinoenamel junction to the surface of the enamel. If each prism is formed by an individual ameloblast this is necessarily so. Fortunate thin sections of decalcified immature enamel show prisms which can be traced through the entire thickness of the enamel. Confusion in this respect is caused by examination of oblique sections in which the alternation of bundles or zones of prisms running in different directions produces the effect so commonly misinterpreted as "gnarled" enamel.

The enamel prisms are not often strictly prismatic in shape in human enamel, but are mostly fluted columns which often have blade-like processes.[9] They are separated by the interprismatic substance except where interprismatic bridges occur. The question arises as to whether there is a prism sheath in addition to the interprismatic substance. At one time[9] I claimed that there is no prism sheath in completely calcified human enamel. I still believe that this is true. Smreker[37] has shown that young enamel exhibits prism sheaths, old

enamel does not. The prism sheath is a prominent feature in dog's enamel, but most of our laboratory dogs are young. The nature of the sheath is not known. Smreker believed that it is the deeper portion of the interprismatic substance contiguous with the prism and that it remains permeable much longer than the rest of the enamel. As stated above, the enamel continues to receive calcium salts for a long time after maturation.[41] The pathway of that transfer could well be by way of the prism sheaths. These, when mineralization is completed, would then become completely calcified.

The multitudinous tests of the permeability of enamel have yielded exceedingly diverse results. Evaluation of these results forces the conclusion that immature or incompletely calcified enamel has some degree of permeability, but that most of the penetration of stains into completely calcified enamel is by way of imperfections, or is caused by the decalcifying action of the staining substance (e.g., silver nitrate).

Imperfections by way of which stains can penetrate the enamel are also possible pathways of attack by caries. They include incremental lines, enamel tufts, lamellae, enamel spindles and granular prisms. The incremental lines, or striae of Retzius, are zones of faulty calcification. They and their outcroppings on the surface of the enamel, the imbrication lines, have been shown to favor the spread of caries.[1]

The enamel tufts are formed at interfaces between bundles of prisms at the peaks of the scallopings of the dentinoenamel junction. They are due to imperfectly formed enamel and are readily permeable to dyes.[18]

The lamellae are thin sheets of organic material which often extend through the entire thickness of the enamel, some of them even into the dentin. Orban[33] differentiated a type which exhibits poorly calcified prisms and interprismatic substance and a type which is a crack formed before eruption and invaded by tissue elements. Miller[32] plotted the location of cracks before decalcification of the enamel and showed that some of the cracks presented the appearance of lamellae after decalcification. Miller also noted their direct continuity with an organic film covering abraded surfaces of the enamel. I believe that most, if not all, lamellae have their origin in cracks which may become permeated by organic matter from the environment of the enamel before or after eruption. Possibly such cracks form in imperfectly calcified regions of the enamel, as Miller[32] suggested, but the fact that lamellae sometimes extend diagonally across the prisms makes difficult an interpretation of zones of imperfection on a developmental basis. The fact that the organic component of some lamellae so closely resembles the probably bacterially produced pellicle over the surface of the enamel[32] and the direct continuity of these organic substances render their common origin probable.

The order in which the parts of the enamel are attacked by acids has been variously reported. Some investigators[6, 25] found that the prisms are dissolved before the interprismatic substance; others[15, 22] that the interprismatic substance is attacked first. Microscopic examination of thin ground sections which are being slowly decalcified by the action of dilute acids run under the cover-glass[9] shows that the interprismatic substance is dissolved first in some specimens, the

prisms first in others. Occasionally both are attacked at the same rate. This variability suggests a difference in quality of the enamel, perhaps associated with its age or degree of maturation or calcification.

The enamel spindles arise chiefly in the earliest formed enamel over the cusps by the penetration of odontoblastic processes through the ameloblastic layer of the enamel organ, as Lams[29] demonstrated. In the formed enamel they are spaces occupied, or formerly occupied, by odontoblastic processes, hence are pathways for fluid penetration into the enamel.

Granular prisms are apparently poorly formed prisms common near the dentinoenamel junction.

In 1926, I[8] called attention to the marked similarity of the probably bacterially produced pellicle found on the enamel of many teeth to the pellicle of the primary enamel cuticle. Such a pellicle could reform, in incubated saliva, in as little as three days after removal by vigorous brushing. Dobbs[16] reported a similar film and speculated on its possible association with the accumulation of acid beneath it. Since then von Korff,[28] Bibby,[3] Manly,[30] and others have dealt with this or a similar pellicle. Vallotton[38] has given an excellent review of the literature. Its association with a bacterial film and the frequent pigmentation have been noted often. It is undoubtedly the same film as that described by Miller[32] and many other early investigators as the enamel cuticle. Manly[30] and Vallotton[38] have noted its keratinous nature. Dobbs[16] and others have mentioned the possibility that it is a "denatured or otherwise altered mucin."

I am informed that my time has elapsed, so I shall leave the association of such a pellicle with the mechanism of dental caries to those who deal with the subject of plaques later in the program.

REFERENCES

1. Barker, F. J.: Importance of Normal and Abnormal Tooth Structures in the Progress of Dental Caries, *J. A. D. A.* **18**: 17-38, 1931.
2. Bevelander, G., and Johnson, P. L.: Odontoblasts and Dentinogenesis, *J. D. Res.* **25**: 381-5, 1946.
3. Bibby, B. G.: A Study of a Pigmented Dental Plaque (Caries), *J. D. Res.* **11**: 855-72, 1931.
4. Blayney, J. R., Wassermann, F., Groetzinger, G., and DeWitt, T. G.: Further Studies in Mineral Metabolism of Human Teeth by the Use of the Radioactive Phosphorus, *J. D. Res.* **20**: 559-63, 1941.
5. Bodecker, C. F.: The Soft Fiber of Tomes, a Tubular Structure; Its Relation to Dental Caries and the Desensitization of Dentin, *J. N. D. A.* **9**: 281-92, 1922.
6. Bodecker, C. F.: The Bacterial Invasion of the Enamel in Dental Caries, *D. Cosmos* **69**: 987-1002, 1927.
7. Bodecker, C. F., and Lefkowitz, W.: Further Observations on Vital Staining of Dentin and Enamel, *J. D. Res.* **25**: 387-99, 1946.
8. Chase, S. W.: The Origin, Structure and Duration of Nasmyth's Membrane, *Anat. Rec.* **33**: 357-76, 1926.
9. Chase, S. W.: Enamel Prisms and Interprismatic Substance, *Anat. Rec.* **36**: 239-58, 1927.
10. Chase, S. W.: A Critical Review of the Controversy Concerning Metabolism in the Enamel, *J. A. D. A.* **18**: 697-710, 1931.
11. Chase, S. W.: Histogenesis of the Enamel, *J. A. D. A.* **19**: 1275-89, 1932.
12. Chase, S. W.: The Nature of the Enamel Matrix at Different Ages, *J. A. D. A.* **22**: 1343-52, 1935.
13. Chase, S. W.: The Interrelation of Maturation and the Histogenesis of Hypoplasia in Dental Enamel, p. 425-32. (In Anderson, G. M., ed. Proceedings Dental Centenary Celebration, Baltimore, Waverly Press, 1940. VII—1061 p.)

14. Deakins, M.: Changes in the Ash, Water, and Organic Content of Pig Enamel During Calcification, *J. D. Res.* **21**: 429-35, 1942.
15. Dietz, V. H.: In Vitro Production of Plaques in Caries, *J. D. Res.* **22**: 423-40, 1943.
16. Dobbs, E. C.: Surface Resistance of Human Enamel to Acid Decalcification, *J. D. Res.* **12**: 581-4, 1932.
17. von Ebner, V.: Über die histologischen veränderungen des Zahnschmelzes während der Erhartung ins besondere beim Menschen, *Arch. f. mikr. Anat.* **67**: 18, 1906.
18. Faber, F.: Das organische Gewebe des menschlichen Zahnschmelzes, *Zeitschr. f.d. ges. Anat.* (Abt. 1) **86**: 1-70, 1928.
19. Gottlieb, B.: Development and Physiologic Purpose of the Organic Matter of Enamel, *J. D. Res.* **22**: 185-90, 1943.
20. Gottlieb, B.: Dental Caries, *J. D. Res.* **23**: 141, 1944.
21. Gottlieb, B.: The Purpose of the Odontoblasts, *J. D. Res.* **25**: 25-7, 1946.
22. Gustafson, Gösta: The Structure of Human Dental Enamel (Göteborg, Elanders Boktryckeri Aktiebolog, 1945), 150 pp.
23. Hevesy, G., Holst, J. J., and Krogh, A.: Investigations on the Exchange of Phosphorus in Teeth Using Radioactive Phosphorus as an Indicator, *Danske Vidensk. Selsk. Biol. Med.* **13**: 34, 1937.
24. Hevesy, G., and Armstrong, W. D.: Exchange of Radiophosphate by Dental Enamel, *J. D. Res.* **19**: 318, 1940.
25. Kanthak, F. F., and Benedict, N. C.: The Effect of Dilute Acids on the Enamel Surface as Disclosed by the Reflecting Microscope, *D. Cosmos* **74**: 439-41, 1932.
26. Kitchin, P. C.: Some Observations on Enamel Development as Shown in the Mandibular Incisor of the White Rat, *J. D. Res.* **13**: 25, 1933.
27. von Korff, K.: Die Enterwicklung der Zahnbeingrundsubstanz der Sänge tiere, *Arch. f. mikr. Anat.* **67**: 1, 1905.
28. von Korff, K.: Die Zusammensetzung und Entstehung der sogenannten nasmythschen Membran und der Cuticula dentis primitiva, *Deutsche Monatschr. f. Zahnh.* **48**: 817, 1930.
29. Lams, H.: Histogenese de la dentine et l'émail de las mammiferes, *Soc. de Biol. Belge, Compte Rendu* **83**: 800-2, 1920.
30. Manly, R. S.: A Structureless Recurrent Deposit on Teeth, *J. D. Res.* **22**: 479-86, 1943.
31. Manley, M. L., and Bale, W. F.: The Metabolism of Inorganic Phosphorus of Rat Bones and Teeth as Indicated by the Radioactice Isotope, *J. Biol. Chem.* **129**: 125-34, 1939.
32. Miller, W. D.: The Presence of Bacterial Plaques on the Surface of Teeth and Their Significance, *D. Cosmos* **44**: 425-46, 1902.
33. Orban, B.: Histology of the Enamel Lamellae and Tufts, *J. A. D. A.* **15**: 305-15, 1928.
34. Orban, B.: The Development of the Dentin, *J. A. D. A.* **16**: 1547-8, 1929.
35. Saunders, J. B., de C. M., Nuckolls, James, and Frisbie, H. E.: Amelogenesis, *J. Am. Col. Dent.* **9**: 107-36, 1942.
36. Sicher, H.: The Biology of Dentin, *Bur* **46**: 121-7, 1946.
37. Smreker, E.: Histologische untersuchungen über den Bau der Schmelzes der Hundzähne, *Ztschr. f. Stomatol.* **28**: 1030, 1930.
38. Vallotton, C. F.: An Acquired Pigmented Pellicle of the Enamel Surface. I. Review of Literature. II. Clinical and Histologic Studies. III. Chemical Studies, *J. D. Res.* **24**: 161-87, 1945.
39. Wassermann, F.: Analysis of the Enamel Formation in the Continuously Growing Teeth of Normal and Vitamin C Deficient Guinea Pigs, *J. D. Res.* **23**: 463-509, 1944.
40. Wassermann, F., Blayney, J. R., Groetzinger, G., and DeWitt, T. G.: Studies on the Different Pathways of Exchange of Minerals in Teeth With the Aid of Radioactive Phosphorus, *J. D. Res.* **20**: 389-98, 1941.
41. Weinmann, J. P., Wessinger, C. D., and Reed, G.: Correlation of Chemical and Histological Investigations on Developing Enamel, *J. D. Res.* **21**: 171-82, 1942.

DR. EASLICK.—Thanks, so much, Dr. Chase, and I am sorry that we rushed you after you worked so hard preparing your paper. As you will note in these programs, which you have there are discussion periods interspersed following each speaker. They are provided for that purpose. And, of course, these discussion periods are arranged for group participation. They are to be used to present a varying viewpoint if anyone so wishes, or to clear up confusing statements, or to ask for more information about any point or any detail presented. I should like to ask, therefore, at this point, are there any people here—is there anyone here who would like to discuss the histological background which Dr. Chase has presented? Dr. Zander?

DR. HELMUT A. ZANDER.—(Tufts College Dental School.) Dr. Easlick, according to the program, this is the only opportunity that we have to discuss the histology associated with dental caries.

DR. EASLICK.—Yes.

DR. ZANDER.—And so I think we should not let Dr. Chase get off too easily; we should try to get a little more into the significance of these features which he presented, the significance to the caries process. And so, the $64 question is "What is the initial caries lesion as it can be seen under the microscope?" What can an histologist tell us about what happens as he interprets what he sees magnified by the microscope as caries attacks enamel? Now, I do not know whether it is fair to ask you this question, but I am sure that you have seen sections of carious teeth, and that other men in this room have seen histological sections of carious teeth, so I think that the initial lesion should be discussed further.

DR. EASLICK.—Thank you, Dr. Zander.

DR. CHASE.—I shall say frankly that I do not know. I really do not know much about that. You probably have read Dietz' paper. He made an apparatus for producing artificial caries and examining the penetration of the acid produced in surroundings which imitated the conditions of the saliva in the mouth. He was able to confirm Williams' old observation, that caries always attacks under a bacterial film. I do not know the mechanism of the acid attack. I just throw up my hands, that is all.

DR. ZANDER.—This information is needed badly. I have seen a few sections, but I am young in this area of research and a lot of men must have seen more than I. After all, that is the basic thing to settle as we go along; later on we should have a definite idea about what happens to the histologic structures of teeth when caries attacks them. I think that his knowledge is so fundamental that we cannot let that question rest.

DR. EASLICK.—Let's hear what Dr. Hill has to say. He also comes from Cleveland.

DR. THOMAS J. HILL.—(Western Reserve University.) I am not going to tell you what happens, I am going to tell you what it looks like, and then you can make your own determination of what happens. The first thing that we see in the attack of caries is that the optical index of the enamel has been changed. In other words, this is the surface of the enamel (indicating on blackboard). Let's assume that it is a gingival cavity or something like that. The first thing that we see will be an area of enamel which becomes, with transmitted light, darker. As yet there is no carious lesion visible, but the area becomes darker. The next thing that we see is that the enamel rods stand out more prominently than they did before, that is, they are just a little bit plainer. On following along such an area we will see that the enamel seems to show what has been called the "old basket weave" or a little checking of the rods (indicating). All of those things happen before an actual lesion begins to occur, and it is from that point that we then begin to get a breaking down of this area (indicating), and the lesion begins. Does that answer your question?

DR. ZANDER.—Thank you very much. While you are there, let me ask you one more.

DR. HILL.—That is the only thing I know.

DR. ZANDER.—What is the relation, if any, between these cracks to the beginning—to the initial carious lesion? Can you find any relationship between the cracks and the initial cares, about which you just told us there?

DR. HILL.—No, not necessarily. It is quite true that very often one will find that, if this area (indicating the darkened area) is quite large, after the section has been made one will find cracks, and that there may be different cracks going that way (indicating), but I think that what is seen is purely a fracture produced during the process of grinding. I am talking about ground not decalcified sections.

DR. DONALD A. WALLACE.—(American Dental Association.) How does this fit in with the theory of organic pathways for caries?

DR. HILL.—This is not my paper.

DR. WALLACE.—I know, but you are on the spot. Would you object to pointing out, if you believe the audience should have it, Gottlieb's mechanism for the initiation of caries?

DR. HILL.—I think that will come up again, Dr. Wallace.

DR. WALLACE.—All right.

DR. EASLICK.—Thank you, Dr. Hill.

DR. HAMILTON B. G. ROBINSON.—(Ohio State University.) I should like to point out—Dr. Hill said it, but I think it should be pointed out again—that this initial area appears white by direct light, and he has sketched the situation for transmitted light, but if one places a direct light on it, it appears white. So clinically these are white spots, but they appear dark under the microscope. There is a confusion of darkness and lightness in this discussion. When histologists say a dark spot, it is what they see with their naked eyes as a white spot.

DR. Hill.—That is right.

DR. EASLICK.—Dr. Taylor?

DR. EDWARD TAYLOR.—(State Dept. of Health, Austin, Texas.) I would like to ask Dr. Chase, are these lamellae, that you mentioned, do they extend through the thickness of the entire enamel to the dentino-enamel junction?

DR. CHASE.—Yes, and they also extend many times, as Faber showed very clearly, way down into the dentin, and that, in my mind, is very good evidence that they are at least in large measure cracks.

DR. TAYLOR.—You say these lamellae are filled with organic matter?

DR. CHASE.—Apparently they are filled with some organic material, that resembles the bacterially produced film from the surface of the tooth and also the pellicle of the enamel cuticle.

DR. TAYLOR.—Is it logical to assume then there is the beginning of caries?

DR. CHASE.—Well, they, of course, have been accused of favoring the penetration of caries. I think perhaps it is pretty well grounded that they do. I do not know anything about caries.

DR. TAYLOR.—I thought you might.

DR. EASLICK.—Dr. Fosdick?

DR. LEONARD S. FOSDICK.—(Northwestern University.) Just for a point of information, I should like to ask Dr. Chase if there is any indication that there is organic material in the enamel, or can it be demonstrated in the enamel—in the intact enamel without any previous decalcification, with the exception of these lamellae?

DR. CHASE.—If I understand your question correctly, you mean, can we demonstrate it without decalcification?

DR. FOSDICK.—That is right, with the exception of the lamellae.

DR. CHASE.—We can demonstrate the content of the enamel spindles in young teeth; we can see that there is something in there. Of course, I think, that it certainly is an odontoblastic process, or the remains of one, but I do not know of anything else that can be demonstrated without decalcification beside the lamellae and the spindles—the contents of the spindles.

DR. FOSDICK.—Would you assume then that there is no organic material there?

DR. CHASE.—I do not think that that is a legitimate assumption because we can recover organic remains from enamel.

DR. FOSDICK.—What I am getting at, I was wondering if that organic material was in the form of calcium salts, say, for instance, a calcium protein that would appear inorganic until it was calcified. Is there anything to indicate that this assumption might be true?

DR. CHASE.—None that I know.

DR. EASLICK.—Having reviewed this much of the pertinent factors of embryology, histology, and physiology, I think that we will have to turn to this still highly controversial area of our problem, "How does dental caries take place?" The first speaker in this area comes to us from the National Institute of Health by way of Chicago's Zoller Clinic, where he completed his graduate work in an investigation of the process of caries. He has been assigned the subject, "Some of the Local Factors in the Development of Cavities—Plaques, Acidity, Aciduric Bacteria, Proteolytic Bacteria." Dr. Stephan, it will be most interesting, I think, to learn how you handle your subject during the next forty-five minutes. Dr. Robert M. Stephan.

SOME LOCAL FACTORS IN THE DEVELOPMENT OF CAVITIES—PLAQUES, ACIDITY, ACIDURIC BACTERIA, PROTEOLYTIC BACTERIA

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FOREWORD

It has not seemed desirable to publish all of the material which was presented at the University of Michigan Conference concerning local factors in dental caries, particularly since most of the data have been published in the JOURNAL OF DENTAL RESEARCH before. Rather, I have tried to bring together those parts which seem to me to suggest the main trends in the development of our present knowledge of the caries process.

R. M. S.

A little more than one hundred years ago, Robertson[1] published the results of critical dental observations which demonstrated that caries begins on certain well-defined "susceptible" surfaces of teeth and then progresses inward toward the pulp. Previously the idea was prevalent that caries arose from within the tooth, as an inflammatory process of systemic origin. Robertson's observations, which have been well substantiated by many investigators since, established that factors which are localized on the tooth surface are of primary importance in the caries process. What has been discovered about the nature and origin of these local factors?

In the voluminous literature on dental caries which has accumulated since Robertson's time, the following observations would seem to be of more than historical significance, in that they have been confirmed and greatly extended by many investigators since they were first introduced:

1. The histological observations by Milles and Underwood in 1881[2] that microorganisms are always present in carious lesions, but not in normally intact tooth substance.

2. The clinical and microscopical observations of J. Leon Williams[3] in 1897, and later by G. V. Black[4] that dense masses of microorganisms (so-called plaques) grow on "caries susceptible" surfaces of teeth.

3. The laboratory observations of W. D. Miller,[5] in 1891, that calcified tooth substance incubated in saliva-food mixtures is attacked only when carbohydrate foods are used and acid fermentation takes place.

4. The bacteriological observations of Kligler,[6] in 1915, that certain types of microorganisms (i.e., lactobacilli) grow frequently on the teeth of individuals with active caries, but infrequently on the teeth of individuals who do not have active caries.

5. Recent epidemiological observations by Dean[7] and also laboratory and animal experiment data by other investigators which show that an inverse relationship exists between fluoride and dental caries.

Together these observations have been interpreted to indicate that the attack on tooth substance in caries is produced by acid fermentation of carbo-

hydrate food material by certain microorganisms growing on or in teeth, and that resistance of teeth to this attack is increased by fluoride in the teeth.

Unfortunately, the question of whether the organic or inorganic material in enamel is attacked first in caries has not been definitely answered because with acid decalcification, such as is employed in the usual histological techniques, the enamel almost completely disappears. It is significant, however, that in caries of the dentin the organic matrix remains after the decalcification produced by the caries process has occurred, and it seems likely that the same would hold true of the enamel. Therefore, on the basis of present evidence, the generally accepted conclusion that the caries process is primarily one of decalcification appears justified. However, there still remain many phenomena of individuals, either with or without active caries, which do not seem explainable on the basis of the aforementioned observations alone and which suggest that other factors, as yet unrecognized, are important in the caries picture.

We may now ask the question, What factors localized on tooth surfaces are capable of decalcifying the enamel? There have been many attempts to formulate an exact mathematical expression, on the basis of a solubility product constant, of the chemical conditions required for solubility or precipitation of calcium salts in bones and teeth. However, the fact that these calcium salts are not present as a single stoechiometric compound but contain significant amounts of other substances within the apatite crystal lattice in addition to calcium and phosphate, has interfered with such a formulation.[8] For the present purpose it is sufficient to point out that theoretically in any solution contacting teeth, a substance which is capable of combining with, and thereby decreasing, the concentration of calcium ions (i.e., citrate, tartrate, some proteins) or phosphate ions (i.e., hydrogen-ions) may produce decalcification of enamel if the substance is present in high enough concentration. Concerning the hydrogen-ion, present evidence suggests that a pH level of about 5.0 or below will produce decalcification of enamel under mouth conditions.

What factors are essential for producing and maintaining an acidity of pH 5.0 or below on tooth surfaces? The lowest pH recorded for saliva is 5.3,[9] and generally salivary secretions are close to neutrality. There seems to be no real evidence to support the belief that saliva itself produces decalcification of teeth. On the other hand, foods have a wide range in pH, and fruits in particular may be quite acid. Experimental studies on laboratory animals[10] have indeed demonstrated that teeth may be decalcified by acid beverages and citrates, but such decalcifications resemble the decalcifications seen in dental erosion in human beings, and do not resemble caries.[10] There seems to be no evidence to indicate that acids directly introduced into the mouth from an external source have any etiological relationship with caries; the decalcifications which such acids may produce generally occur on those tooth surfaces which are not susceptible to caries.

Microbic plaques remain as a possible source of acid production in the initiation of caries. In a previous publication[11] it was observed that microbic plaques produce a rapid drop in pH when glucose is ingested in the mouths of caries-free individuals as well as in those with active caries, but the plaques in

the mouths of caries-free individuals did not drop below pH 5.0, whereas in the mouths of caries-active individuals the pH sometimes reached 4.0. This observation suggests that the microbic flora growing in the plaques of caries-active individuals differs from the microbic flora in plaques of caries-free individuals. This difference appears not to be so much in the rate at which acid is produced at pH levels above 5.0 but rather in the relative ability of plaques in caries-active individuals to continue production of acid below 5.0 and the relative inability to remove the acid produced, as compared with plaques of caries-free individuals.

Other types of differences between microbic plaques have been noted, such as the observations of Kligler,[6] Bunting,[12] Hemmens,[13] and others that relatively greater numbers of lactobacilli can be grown from the plaques of caries-active individuals than from plaques of caries-inactive individuals, and also the observation of Blayney, Kesel, and Wach[14] that relatively more short-rod forms can be seen microscopically in smears of plaques from teeth with beginning caries than from teeth which remain caries-free.

In another study[15] it was found that different types of oral microorganisms, when studied with in vitro pH experiments in pure culture and at high cell concentrations which approached the cell concentration in plaques, gave pH curves with glucose which in many ways simulated the pH curves produced by plaques with glucose in situ. Particularly it was observed that with most microorganisms, as with plaques, pH changes were rapid. Also, with a given amount of glucose to metabolize, some microorganisms produced and maintained pH levels below 5.0, whereas with the same amount of glucose other microorganisms did not produce or maintain pH levels below 5.0. Thus, some microorganisms (i.e., lactobacilli) produced pH curves which in many respects resembled pH curves produced by the plaques of caries-active individuals, and other microorganisms (i.e., staphylococci) produced pH curves which resembled more the pH curves produced by plaques in caries-free individuals.

While a comparison between these in situ experiments on plaques[11] and the in vitro experiments with concentrated masses of oral microorganisms[15] is not completely valid as a result of differences such as those produced by salivary flow in situ, nevertheless, in each case the data suggest that it is not so much the rate at which acid is produced which distinguishes the plaques and oral microorganisms associated with caries activity from those not associated with caries activity, but rather the ability to continue to produce acid at low pH levels and the relative inability to consume the acid thus produced.

This situation raises the question in regard to whether there are any significant differences in the enzymatic pathways by which food substances are metabolized by the microorganisms on teeth with active caries as compared with the microorganisms on caries-free teeth. The series of well-known enzymatic steps in glycolysis has been shown to be present in many microorganisms.[16] On the basis of rapidity of acid formation from glucose, it appears that neither the microorganisms in plaques in caries-free mouths nor the microorganisms in plaques in mouths with active caries are deficient in glycolytic ability. This conclusion is in agreement with the findings of Miller and Muntz[17] on lactic

acid production from carbohydrates by dental plaque material, but it is contrary to the hypothesis postulated by Fosdick[18] that oral microorganisms such as lactobacilli are deficient in certain glycolytic enzymes, and require additional enzymes as furnished by yeasts for rapid production of acids. As a matter of fact, in pH experiments in which high concentrations of lactobacilli and yeast cells were mixed together, the acidity produced from glucose was much less than that produced by lactobacilli alone.[15] This admixture with yeasts actually prevented lactobacilli from reaching the low pH levels associated with caries. It seems probable from this evidence[11, 15, 17] that the chief difference in carbohydrate metabolism between oral microorganisms associated with active caries and those not associated with active caries occurs in the enzymatic steps which occur after the formation of pyruvic and lactic acids. Thus oral microorganisms, such as lactobacilli, in plaques permit a more prolonged accumulation of acid intermediates of metabolism (i.e., lactic acid), whereas many other types of oral microorganisms in plaques either relatively quickly oxidize the acid intermediates to carbon dioxide and water or else, like yeasts, reduce them to nonacid substances such as alcohol. In the search for new methods to prevent dental caries there is a need for a much more comprehensive knowledge of the metabolic activities of different oral microorganisms than exists at the present time.

REFERENCES

1. Robertson, William: A Practical Treatise on the Human Teeth, ed. 2, London, 1939, Hayward and Moore.
2. Milles, W. T., and Underwood, A. S.: An Investigation Into the Effects of Organisms Upon the Teeth and Areolar Portions of the Jaws, *International Medical Congress, Transactions (7th)*, vol. 3, London, 1881, Kolckmann, pp. 523-529.
3. Williams, J. L.: Studies of Enamel, *Dental Cosmos* **39**: 269-301, 1897.
4. Black, G. V.: Operative Dentistry, vol. I, 1908, Chicago, Medico-Dental Publishing Co.
5. Miller, W. D.: The Micro-organisms of the Human Mouth, Philadelphia, S. S. White Dent. Mfg. Co., 1890.
6. Kligler, I. J.: A Biochemical Study and Differentiation of Oral Bacteria With Special Reference to Dental Caries, *J. Allied Dent. Soc.* **10**: 141-166, 282-330, 445-458, 1915.
7. Dean, H. T.: Dental Caries and Fluorine, A.A.A.S., 1947, Washington, D. C.
8. Eisenberger, S., Lehrman, A., and Turner, W. D.: The Basic Calcium Phosphates and Related System, Some Theoretical, Practical Aspects, *Chem. Rev.* **26**: 257-296, 1940.
9. Babkin, B. P.: The Physiology of Salivary Glands. In Gordon, S. M., ed. *Dent. Science and Art*, Philadelphia, 1938, Lea & Febiger.
10. McClure, F. J.: The Destructive Action, in Vivo, of Dilute Acids and Acid Drinks and Beverages on the Rats' Molar Teeth, *J. Nutrition* **26**: 251-257, 1943.
11. Stephan, R. M.: Intra-oral Hydrogen-ion Concentrations Associated With Dental Caries Activity, *J. D. Res.* **23**: 257-266, 1944.
12. Bunting, R. W., and Parmelee, Faith: The Role of Bacillus Acidophilus in Dental Caries, *J. A. D. A.* **12**: 381-413, 1925.
13. Hemmens, Elizabeth S., Blayney, J. R., and Harrison, R. W.: The Microbic Flora of Bacterial Plaques Removed From Carious and Noncarious Dental Enamel, *J. D. Res.* **20**: 29-38, 1941.
14. Blayney, J. R., Kesel, R. G., and Wach, E. C.: Dental Caries, I. New Method of Studying Bacterial Plaque, *J. D. Res.* **15**: 236, 1936.
15. Stephan, R. M., and Hemmens, Elizabeth S.: Studies of Changes in pH Produced by Pure Cultures of Oral Micro-organisms, *J. D. Res.* **26**: 15-41, 1947.
16. Barron, E. S. G.: Mechanisms of Carbohydrate Metabolism: an Essay on Comparative Biochemistry, vol. III, New York, 1943, Interscience Publishers, Inc., pp. 149-189.
17. Miller, B. F., Muntz, J. A., and Bradel, S.: Decomposition of Carbohydrate Substrates by Dental Plaque Material, *J. D. Res.* **19**: 473-478, 1940.
18. Fosdick, L. S., and Wessinger, G. D.: Carbohydrate Degradation by Mouth Organisms, II. Yeast, *J. A. D. A.* **27**: 203-212, 1940.

DISCUSSION

DR. HELMUT A. ZANDER.—Dr. Gottlieb states that without organic invasion roads, the lamellae, bacteria do not have an opportunity to get a foothold on the tooth surface, and we will have no plaques and no caries. Do you believe that it is necessary for bacterial plaques to start from these lamellae?

DR. STEPHAN.—I think that we might cite G. V. Black's work on "gelatinous" plaques. He showed that some microorganisms isolated from plaques formed colonies which adhered very tightly to the wall of glass test tubes in which they were grown. I think that there is nothing comparable to lamellae in glass test tubes. It seems to me that plaques are probably not dependent on lamellae to retain their hold on teeth.

DR. DONALD A. KERR.—The thing, I think, that is against the lamellar theory of caries as the only source of initial caries is that nature isn't too accommodating to put lamellae at the contact points of teeth, or the point where we usually see caries. The lamellae occur on all surfaces of the teeth, and we find caries localized to particular surfaces. I think that lamellae do not always occur at that point.

DR. WALLACE ARMSTRONG.—I have a question on quite a different subject from the Gottlieb and Miller theory; it has to do with the mechanism by which the plaque can promote the carious lesion. You understand that this is a means by which a high concentration of hydrogen-ions is produced locally through bacterial action presumably in the usual case from carbohydrate. Well, I have always been impressed with the rather high buffer capacity of the saliva. And for my own information I should like to know if there is any reason to suppose that there is some differential permeability of the plaque material which would at the same time allow the substrate to diffuse into it but not the buffer of the saliva, and which would also prevent the outward, in a sense, diffusion of the hydrogen-ions in the interior cavity.

DR. STEPHAN.—I think this is the idea that Lothrop and Gies had when they tried to demonstrate a membrane of precipitated mucin in "mucinous plaques." Also Black had this idea in working on "gelatinous plaques," but so far as I am aware they could not confirm their ideas by experimental evidence. The plaque is a mass of microorganisms, and as such it is a whole series of membranes, one might say, since each microorganism has its own membrane. It seems to me that the spaces between the microorganisms would permit diffusion, certainly in a very crooked path to avoid the microorganisms, but nevertheless diffusion through whatever material is between the microorganisms. I think that acid is formed rapidly enough by plaques so that it is not necessary to postulate a membrane effect to account for the acidity in caries.

DR. EASLICK.— I should like to tell you that the subject of Dr. Gottlieb's hypothesis and treatment is not dead, that it will be revived because Dr. Zander has the subject of the use of silver nitrate as a preventive technic, or as a control technic of dental caries. You will be hearing more about Dr. Gottlieb's work later.

Again, I should like to remark that almost invariably, when I discuss dental caries with a lay group, someone propounds the question, "Well, there was my Uncle Ned or grandfather or cousin Sarah who lived until he was seventy-eight years old. He never brushed his teeth; he never went to the dentist; he ate anything he liked; and he died with practically all of his teeth intact. How do you explain Uncle Ned's teeth?"

I am happy, for once in my life, to pass this old Uncle Ned question on to young Ned Williams who has been assigned the topic, "Factors in Immunity and Susceptibility to Dental Caries." Dr. Williams comes to us most recently from the University of Pennsylvania where he just became an Assistant Professor of Bacteriology. He, too, came by the way of the Zoller Clinic in Chicago where he pursued his graduate work. Dr. Williams, you have the next forty-five minutes, and I hope everything that I have said about you has been correct.

DR. WILLIAMS.—The usual discussion of a title such as mine touches upon so many different types of subjects related to dental caries, that it is scarcely possible to do justice to all of them. Even if I attempted such a discourse with you I would appear to be attempting a summary of all of the topics that will be covered here this week. Therefore, I am limiting my discussion to a narrower field of factors which seem to be involved in immunity to dental caries. Before beginning, it would be advisable for us to consider the word immunity and discuss what it implies.

FACTORS IN IMMUNITY AND SUSCEPTIBILITY TO DENTAL CARIES

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Immunity may be defined as a state of resistance to the development of a disease. If the disease is caused by an infectious agent then the immunity may be due to substances in the blood or in the tissues which assist in destroying the organisms. These substances are called antibodies, and the degree of immunity to an infectious disease is somewhat dependent upon the concentration of these antibodies in the blood and in the tissues. Dental caries is unique in this respect since it cannot strictly be described as an infectious disease, even though bacteria play a role in the process, and thus one would not expect to find antibodies participating directly in immunity to it. An infectious disease is usually initiated after invasion of the tissues, but in dental caries the tooth is usually invaded *after* the enamel tissue has been damaged. The protective substances or antibodies would have direct access to the invading organisms of the infectious disease, but in dental caries access of any such substances to the causative organisms would be very indirect. On the other hand, human beings who are immune to caries demonstrate a resistance which we have not been able to explain, and many authors have suggested that an immunological principle, protective or inhibitory substance, or antibody is active either through the blood or saliva.

Most of us are agreed that bacteria have an important role in caries, but just which bacteria are most important is not yet entirely clear, although available evidence points heavily toward the lactobacilli. But then there are all kinds of lactobacilli, just as there are all types of pneumococci, and we have not yet determined just which specific lactobacilli are involved. In spite of this uncertainty much knowledge has been gained by studying these microorganisms, and I would like to present for your consideration the various reports that have appeared in study of protective or inhibitory substances in the blood.

Several years ago the group here at the University of Michigan were interested in determining tests for susceptibility to dental caries. They made a filtrate from a collection of cultures of lactobacilli and used that filtrate in a skin test in individuals to see what the reaction would indicate insofar as susceptibility to dental caries was concerned. They found that those persons who reacted to the filtrate, following intradermal injection, did not have lactobacillus antibodies in their blood sera, while those whose skin tests were negative did have lactobacillus antibodies in their sera. The determinations for presence or absence of antibodies in the sera of these persons were made by using the agglutination test, a widely used test for detection of specific antibodies in blood sera. Parenthetically, the principle of the agglutination test is that an even sus-

pension of bacteria is made and kept so that these bacteria will remain evenly suspended. Then various dilutions of the serum are made beginning, perhaps, with one part of serum plus one part of saline, and so forth. Parts of the even suspension of organisms are mixed with each dilution of the serum and the tubes are incubated. The highest dilution of serum in which there is no longer an even suspension of organisms is called the titer, or is representative of the strength of the antibodies in that serum.

Correlated with these findings, the persons with antibodies in their sera were immune to caries, and lactobacilli were absent from cultures made of the salivas. The exact opposite was found with the persons who did not have antibodies in their blood stream. Note that here is an unsuspected parallelism between the presence of antibodies in the blood stream and immunity to dental caries. Cultures of lactobacilli were prepared for a series of injections into persons whose sera did not contain antibodies in order to determine whether antibodies could be stimulated. The suspensions for the injections included a rough and a smooth strain of lactobacillus. When we speak of rough and smooth organisms in bacteriology we usually mean the colony types of the organisms on an agar plate. The edges are smooth; the surface of the colony is smooth, and so forth. Consequently we call them smooth, whereas the rough organisms on the plate may have any distortion of this type of colony. This is not an exact definition of the difference, but is sufficient for our purpose.

When the injections were made into the subcutaneous tissues of persons with these kinds of bacteria it was soon found that the rough type of organism was leading to sterile abscesses in the tissues, but those persons who received the smooth organisms did not have any reaction. Blood sera, obtained at this time, were measured for antibodies by the agglutination test. It was found that the sera from persons with abscesses had an increased antibody concentration against lactobacilli, whereas those without abscesses had no appreciable change in lactobacillus antibody concentration. Saliva was cultured from these persons ten days after the last injection, but because of the short observation time it was impossible to draw any conclusions from those tests as to the effect of this increased antibody titer on the lactobacilli in the mouth. The line of approach suggested by these studies is quite interesting because the findings indicated that blood antibodies may have a role in immunity to dental caries, but the results did not show, for the time was too short, whether the antibodies affected lactobacilli in the mouth.

A period of nine years elapsed before another report, concerning this type of study, described injections of lactobacilli into persons in order to determine what the effect of increased antibody in the blood stream might have on lactobacilli cultivable from the mouth. Canby and Bernier had been studying lactobacilli for several years and became interested in developing a suspension of lactobacilli which could be injected without the development of abscesses as described previously. They selected certain strains of lactobacilli by the very tedious process of cross-agglutination tests. Cross agglutination is a series of

agglutination reactions which concern several different kinds of lactobacilli insofar as we can differentiate the different kinds, and anti-sera, prepared in rabbits, aganst those specific cultures. Then one organism is used and tested against each one of the sera; then the same thing is repeated with each organism and all the sera, and so forth. The purpose was to find whether these organisms had anything in common, and second, whether one organism had substances which were present in all of the sera. In other words, if one lactobacillus reacted with all of the sera and other lactobacilli only reacted with two or three, it would appear as though the first organism could stimulate antibodies against any of the others. In this way Canby and Bernier attempted to get an organism which would produce antibodies against as many lactobacilli as possible. They used a different injection method than did the group at Michigan, and after determining the numbers of lactobacilli in the saliva of volunteers and obtaining blood samples, began injecting the lactobacilli intradermally. Note that the intradermal route was employed instead of the subcutaneous.

The rationale for the use of the intradermal route was based on the reported findings of others concerning immunization procedures against typhoid fever. No severe reactions were noted which would necessitate stopping the injections. The smallest number of injections given was nine, and the greatest number was forty-six, but even so, the increase in the antibody concentration of the blood stream was not great. It is well known that the subcutaneous injection of material to stimulate antibody is much the preferable method. The salivas from the various volunteers were cultured for lactobacilli four to ten days after the last injection and it was noted that in almost every instance the average numbers of lactobacilli in the mouth had decreased after the injections in contrast to the average numbers of lactobacilli that had been found prior to beginning the injections.

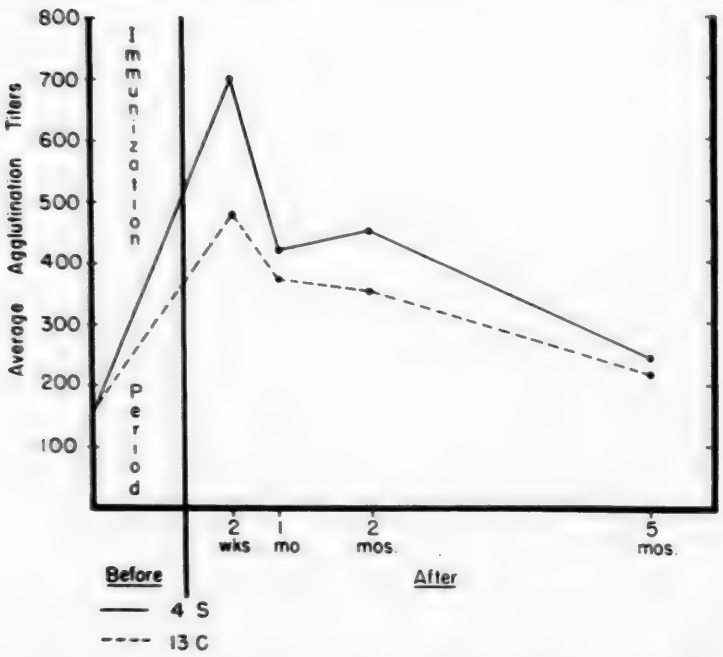
The findings reported by these workers added much impetus to this type of study, and they strongly suggested that further work should be undertaken with controls. Their experience with lactobacilli convinced them that these organisms are not at all homogeneous in their antibody-producing properties, and they asked for a more complete study of the organisms. The war interfered with the work of these men and others were asked to continue it.

The follow-up on this preliminary report came in 1944 when the results of our studies were described. Because of the great number of men available it was possible to expand the project materially and also to add a group of controls. Suspensions for injection purposes were prepared from cultures which had been selected in the same manner as that of Canby and Bernier. However, we resumed the subcutaneous method of injection, as cited before, because it is well known that this method leads to a greater production of antibodies. A great number of prebreakfast salivary samples were obtained from the entire volunteer group prior to beginning the injections so as to give a representative lactobacillus count for each person. All were bled to obtain serum; then the group was divided into two parts, one serving as a control. Injections were given at weekly intervals for four weeks—the same as had been done at Michi-

gan—one arm receiving a living suspension of a mixture of two lactobacillus strains, the other a heat-killed suspension of the same mixture. Preliminary tests had indicated that this method of immunization was more effective than when killed organisms were given alone. Salivary counts and sera were obtained at regular intervals from the injection and control groups beginning two weeks after the last injection and continuing through five months after completion of vaccination.

Figure 1.

Average Agglutination Titers Of Persons Having Reductions In Average Salivary Counts Which Were Greater Than Could Be Accounted For On Basis Of Chance.



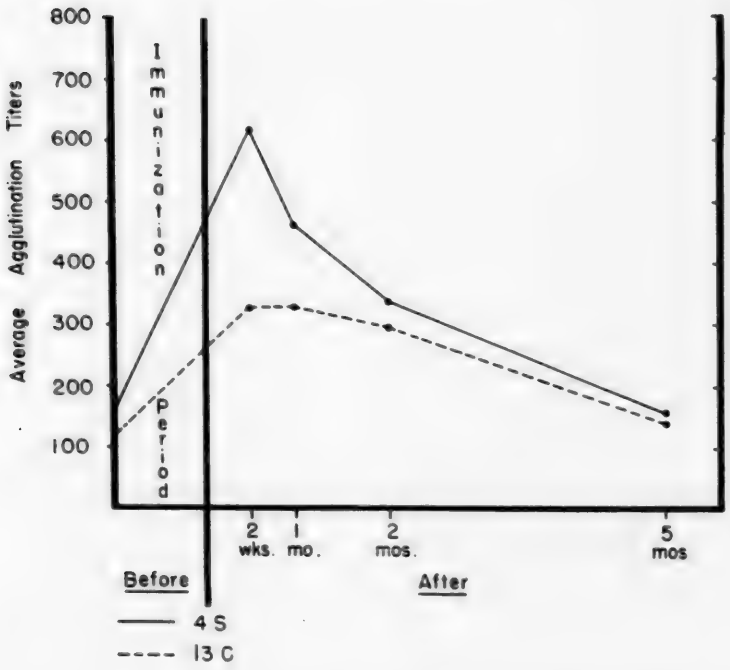
There were no reactions of a local or general nature following any of the injections. Sera were tested by the agglutination reaction with the organisms that had been used for injection purposes and the highest titers were found generally two weeks after the last injection. The titers then decreased gradually until they were near the "normal" level at five months. Control titers remained constant.

Fig. 1 will give an idea of the course and the titer of these antibodies in the blood stream. The average agglutinin titers before the injections show that very few were below 100; most of them were close to 200, being a dilution

of 1 to 200. Two weeks following the immunization the antibodies against this particular strain, represented by the solid line, were in the neighborhood of 700, whereas to the other organism the titers were around 500. And then, as you can see, they gradually came downward. Fig. 2 shows the results on another group of the injected people, and you note that the peaks are nearly the same, although they are somewhat lower. Fig. 3 shows the results on the control group.

Figure 2

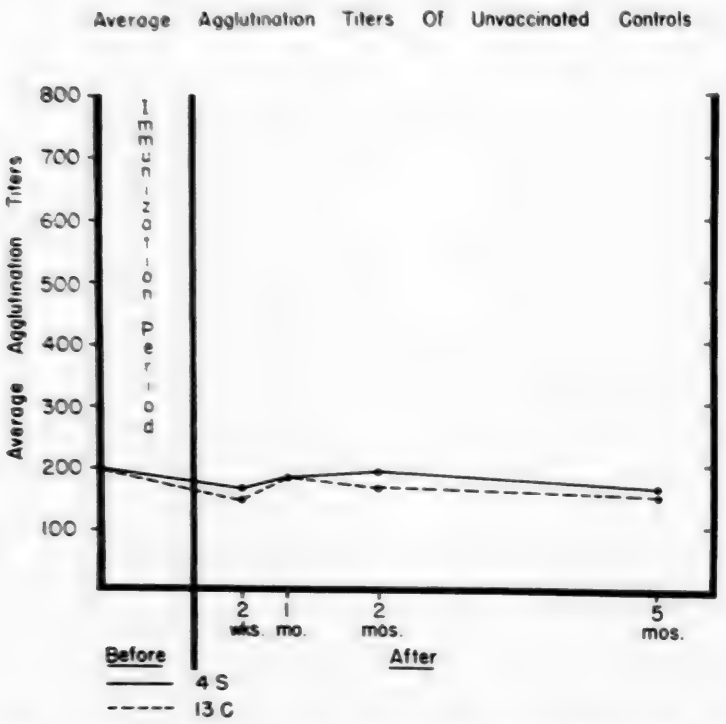
Average Agglutination Titers Of Persons Having Changes In Average Salivary Counts Which Were Within Limits Of Chance Variation



The data on counts for lactobacilli were analyzed by statistical methods because arithmetical averages have little significance unless there is some analysis of the figures used in making up the averages. Almost everyone who has done counts of lactobacilli from saliva has noted that there is tremendous variation in the numbers found even when a single dilution is duplicated. The results of the analysis indicated that fifteen of twenty persons, or 75 per cent, in the immunized group had apparent reductions in average salivary counts. The statistical analysis demonstrated that only five of the twenty, or 25 per

cent, could be regarded as being reductions not due to chance. Table I gives an indication of that. The injections may have had some effect on the salivary counts for lactobacilli in this group. But it can be seen that great variation was obtained in these prebreakfast salivary counts. The counts in Table II were from persons with changes in counts within the limits of chance variation. I should like to point out that the blood sera titer are represented in Fig. 2 and it is noted that the titers were not much lower than in Fig. 1.

Figure 3.



From Table III it may be seen that although the counts for the control group were somewhat lower, and any changes occurring in this group were all due to chance.

The blood serum titers of the five people indicated in Fig. 1 had decreases in their counts that we believe were not due to chance. Fig. 2 shows the blood serum titers of the people who changed lactobacillus counts apparently as the result of chance. The antibody titers in the blood stream are high enough so that if there were effects in the preceding group we would have expected effects in the second group.

These results seemed to indicate that the immunization as performed had no consistent effect on the numbers of lactobacilli cultivable from the oral cavity. No claim is made that this work is the final answer to the approach first sug-

TABLE I

PERSONS HAVING REDUCTIONS IN SALIVARY COUNTS WHICH WERE GREATER THAN COULD BE ACCOUNTED FOR ON THE BASIS OF CHANCE

	BEFORE			AFTER					
				TWO MONTHS			FIVE MONTHS		
VOL	AVERAGE	HIGHEST	LOWEST	AVERAGE	HIGHEST	LOWEST	AVERAGE	HIGHEST	LOWEST
ARA	88*	219	36	30	60	4	215	343	106
MAN	1424	6100	195	173	320	25	238	508	20
REE	814	2500	7	13	18	5	29	33	25
ROS	725	2300	25	245	540	109	—	—	—
SCO	161	434	26	56	127	13	56	147	23

TABLE II

PERSONS WITH CHANGES IN COUNTS WITHIN LIMITS OF CHANCE VARIATION

	BEFORE			AFTER					
				TWO MONTHS			FIVE MONTHS		
VOL	AVERAGE	HIGHEST	LOWEST	AVERAGE	HIGHEST	LOWEST	AVERAGE	HIGHEST	LOWEST
AND	118	386	4	106	254	35	29	64	4
BRA	562	4000	53	332	892	202	426	668	118
CAR	722	6112	16	87	254	11	26	56	2
CUR	2256	9840	80	295	508	100	373	420	217
DIE	49	135	4	53	81	5	—	—	—
GRE	169	480	15	147	381	30	69	212	8
GOL	105	180	24	161	381	31	38	95	16
HAI	411	1588	2	8	25	2	4	8	2
KAU	69	127	16	80	145	32	56	184	1
KLE	5774	13580	1000	6223	10160	2500	2600	3360	2000
LEY	238	812	40	45	101	7	56	110	7
SUB	744	5000	11	311	761	80	1140	2540	200
MAR	660	1271	24	46	190	10	28	57	12
PER	91	728	36	50	181	24	—	—	—
WAT	299	524	13	309	1016	60	—	—	—

TABLE III

CONTROLS—ANY CHANGES WERE WITHIN LIMITS OF CHANCE VARIATION

	BEFORE			AFTER					
				TWO MONTHS			FIVE MONTHS		
VOL	AVERAGE	HIGHEST	LOWEST	AVERAGE	HIGHEST	LOWEST	AVERAGE	HIGHEST	LOWEST
AHA	14*	27	8	10	15	2	4	9	500
BUR	48	133	12	68	81	15	—	—	—
CAH	670	2869	59	533	254	34	51	127	14
FRA	299	734	90	333	1164	200	—	—	—
GUY	20	64	3	16	50	2	10	15	6
HEL	60	294	3	37	127	10	15	23	9
JOH	79	254	21	54	127	10	—	—	—
LAM	12	17	2	9	13	6	—	—	—
PAY	55	194	2	38	76	11	26	35	15
STO	131	327	26	147	386	46	151	343	31
SUS	298	899	20	233	992	25	29	76	10
VOG	313	576	23	259	508	118	—	—	—
PAR	95	533	6	97	512	5	—	—	—

*All figures given are in thousands.

gested by Jay and his associates because, as mentioned earlier, so little is known about the lactobacilli that it is quite possible that the strains of lactobacilli employed may not have been the best ones. The strains used in these tests were of the smooth type, and although increases in antibody concentrations were obtained, it is possible that other strains may have an entirely better effect in antibody production.

Recently a study on rats was reported by Parsons, McCollum and Frobisher who used vaccines containing lactobacilli and acidogenic cocci. They did not find any effect on the numbers of lactobacilli or cocci cultivated from the teeth of the rats. No agglutination tests were reported. Jay and his associates reported injection of rats in 1933 but could not find antibodies in the blood sera.

In a report about to be published, the beginning of an antigenic analysis of lactobacilli isolated from saliva is to be described. This method is used to determine how various lactobacilli differ in their production of antibody. It is similar to selecting the orthodontists, oral surgeons, or periodontists from a group of dentists whose specialties are unknown. In this way it is hoped that it will be possible to make the identification of lactobacilli more reliable, to determine just which lactobacilli are important in the carious process, and possibly to find the kinds or types of lactobacilli which are responsible for the antibodies existing in the blood sera of persons immune to dental caries. It was interesting in this work that many of the rabbits which were used to obtain the sera were found to contain antibodies for some lactobacilli prior to any injections, and these lactobacilli appeared specifically to remove this antibody from the serum. When the serum and bacteria were mixed and the bacteria removed, the serum no longer reacted with the lactobacilli. Not all strains of lactobacilli would remove this antibody from the "normal" rabbit serum. Thus, it is possible that if this component is extended and studied further, it may be found to be a naturally existing or a naturally acquired antibody. If we, for the moment, translate this idea to human beings in whom antibodies for lactobacilli have been found, it is possible that such an antibody may exist there, also. Thus, it is necessary that we determine what strains of lactobacilli are most closely associated with dental caries before attaching too much importance to antibodies against lactobacilli that exist in human beings with or without dental caries.

SUMMARY

In summary, I have attempted to present a series of studies which have approached immunity to dental caries from a very different and perhaps complicated viewpoint. They seem to suggest that we cannot regard dental caries as an entirely local process which may not be affected by the bodily processes. They also indicate that inhibitory processes in the blood stream may be involved in the control of dental caries, although how is a question as yet unanswered. It is becoming more evident each year that there is a growing need for more studies on the fundamental problems underlying and associated with dental caries, and I believe that the trend toward accomplishment of this goal will be apparent to you during this week of meetings.

DISCUSSION

DR. EASLICK.—Thank you very much, Dr. Williams.

The afternoon session is now open to discussion of this "Uncle Ned" question which I raised. Would anyone like to discuss Dr. Williams' paper? Dr. Knutson?

DR. JOHN W. KNUTSON.—I would appreciate it if Dr. Williams would take the time to show the first slide again, and explain the antibody titers in compiling those figures.

DR. WILLIAMS.—The antibody titers were obtained by the use of the agglutination test. A simple arithmetic average of the titers from the various volunteers in each group was used to give us the points on the graph, both before and after the immunization had been completed. This particular graph (Fig. 1) was made from that group of persons in which we found that the counts had been changed after the completion of the injections, and we could not explain that change as being due to chance.

DR. KNUTSON.—What proportion of your total group does that represent?

DR. WILLIAMS.—These were five of twenty. There were five people in this chart.

DR. KNUTSON.—Thank you.

DR. EASLICK.—Dr. Armstrong?

DR. WALLACE D. ARMSTRONG.—May I ask another question? In trying to understand how the agglutination antibodies might inhibit the process, one would suppose that if the antibodies were in saliva, that in that way this effect would come about. Now, the saliva is, of course, secretion which is derived from the blood and contains many substances present in the blood plasma, although, true enough, frequently in vastly different concentrations. Is it technically possible to demonstrate these specific antibodies in the saliva, and if so, did you find an increase in the antibody concentration in the saliva paralleling the time and the blood?

DR. WILLIAMS.—Dr. Armstrong, we did not do any studies on the saliva for antibodies. That was quite unfortunate.

DR. ARMSTRONG.—Is it possible to do it?

DR. WILLIAMS.—It has been done. Dr. Jay has done it, and some of the British people have done it, but all have reported that the antibodies in saliva were very low in concentration.

DR. ARMSTRONG.—You need someone with a very low lactobacillus count, or else you would get agglutination adsorption in the saliva?

DR. WILLIAMS.—That is possible and is a difficulty in this problem. You would really need to obtain the saliva from the salivary ducts before it came in contact with the flora of the mouth in order to make sure that the saliva had nothing removed from it by adsorption by susceptible bacteria of the mouth.

DR. EASLICK.—Dr. JAY?

DR. PHILIP JAY.—I was just going to remark that, as a rule, individuals who had the agglutinins in the serum had negative lactobacillus counts.

DR. WILLIAMS.—Dr. Jay, if I may interrupt, I think what Dr. Armstrong meant was that there may be other organisms there which might have related antigens.

DR. ARMSTRONG.—That was the point I was laboring to bring out.

DR. WILLIAMS.—There may be related antigens which might remove such an antibody from the saliva even though no lactobacilli were present. Antibodies to other infectious disease agents have been found in the saliva.

DR. EASLICK.—Dr. Arnold?

DR. FRANCIS A. ARNOLD, JR.—I would like to ask Dr. Williams how he can explain, if he can, the fact that individuals who are caries free and have no lactobacilli in their mouth are the individuals with a high agglutinin titer? How did they develop the agglutinin titer in their blood stream if they were never infected with lactobacillus?

DR. WILLIAMS.—I don't know. How did the rabbits get it? One can think of many possibilities, and much has come out in the antigenic analysis. It is difficult to determine where these things arise. It takes a lot of time and work to figure them out, but even then they may not mean anything.

DR. EASLICK.—Dr. Earle?

DR. JACK M. EARLE.—How many specific antigens were found in the *Lactobacillus odontolyticus?*

DR. WILLIAMS.—Well, Dr. Earle, I didn't test that particular organism. All the ones I had I called "lactobacilli." There are so many names for lactobacilli that I felt it was important to refer to them as a general group rather than as a specific one. Among the lactobacilli we have worked with so far we have found about three strains which have three or more components within them.

DR. EARLE.—Then, you are getting cross agglutination in more than one?

Dr. WILLIAMS.—Yes, if all those components are present in the organism, then it will cross with that particular component in the serum.

DR. EARLE.—Yes.

DR. EASLICK.—Dr. Jay, did you wish more discussion?

DR. JAY.—Not particularly, but Dr. Arnold's question suggests a thought that was puzzling when we wondered how agglutinins were present in the blood sera of those who had no lactobacilli. We know that the nursing infant has a bacteriological flora that is predominantly gram positive, and undoubtedly a great many lactobacilli are present in the intestinal tract at the time. It may be barely possible that agglutinins were produced at that time and persisted.

DR. WILLIAMS.—Dr. Jay, I am bothered about the same thing. The thing that I have come to assume is that if this thing means anything at all, there may be some sort of anamnestic reaction involved. The organisms that we have used may not be the right ones antigenically; therefore, if we are able to match up the kind of substance that is in the serum with an organism which contains that component or other components, then it may be possible, by selecting those particular bacteria, to keep the antibody titer at a high level over a longer period of time.

TUESDAY, SEPTEMBER 9

Morning Session

DR. EASLICK.—In our week-long program, before we take up the caries control measures that are available, we have a third speaker in the group who has been selected to analyze critically the caries process itself. Dr. Hamilton B. G. Robinson is this speaker. He comes from Ohio State University where he has an appointment as Professor of Oral Pathology and Diagnosis. The subject that has been assigned to him is "The Effect of Systemic Conditions on the Caries Process—Pregnancy, Endocrinopathies, Osteomalacia, Emotional Disturbances, and Others." Dr. Robinson, you may have the first forty-five minutes this morning.

DR. ROBINSON.—We are going to talk about dental caries. The first thing we should do is to find out what dental caries is. I believe that we have not yet discussed dental caries as a process. I would like to give you my idea of the carious process. This is not an original idea but is merely an accumulation of material going back to W. D. Miller and even earlier. To Miller's work we have been able to add, bit by bit, other pieces of information. The man who has come closest to being able actually to watch the carious process, I believe, is Victor Dietz, who devised a sort of an artificial mouth with a tooth in it which could be observed under the microscope while the carious process was active.

Caries begins on the surface of a tooth, and the first thing that we would see would be an attachment of threadlike organisms to the surface of the tooth. This mass of microorganisms would become denser and denser, and finally it would become what we have termed a "plaque." The plaque is a mass of microorganisms that accumulates on a given surface of the tooth. That plaque will become a place in which other bacteria can live. These new bacteria may be acidogenic bacteria capable of producing acid; they may be lactobacilli; they may be streptococci; they may be staphylococci. Many different forms of bacteria may take up their habitat in the plaque. The plaque becomes a microuniverse, a sort of mixed colony. It is a place where there are threadlike organisms, coccal organisms, rodlike organisms, all living together just as we are living with other human beings and other forms of life.

This microuniverse is living inside of the oral cavity, but the oral cavity is outside of the body. The oral cavity merely represents the upper end of a tube that is outside of the body, because the body is the part between the gastrointestinal tract and the surface of the body. The microorganisms in this plaque live on the same food that we live on. When we take in food, they live on that food, not on our tissues. It is simply an accidental relationship that they may do harm because the acid-producing organisms live on carbohydrate. Carbohydrate gets into the plaque, this little universe, and the carbohydrate is utilized by the bacteria. In the process of their metabolism these organisms produce acid and that is where an unfortunate situation arises. They are producing acid on the surface of a substance which can be dissolved by acid; that is, on the surface of enamel. Therefore, the enamel begins to dissolve and we have the initiation of the carious process.

That is rather a simple explanation of what takes place. The carbohydrate, coming in, is changed over to an acid by the metabolic process of this bacteria. Caries is not an infectious disease in the general sense of infectious diseases. When we think of disease, generally, we think of bacteria living in the tissues or living in an organ, but these bacteria just live in the mouth. They aren't mad at anybody; they just take up their habitat in the oral cavity and, by accident, their products lead to a destructive reaction. This is where we run into difficulty because we just can't get our thought processes away from infectious disease in the same sense as other bacterial diseases. We think of bacteria getting into tissue and maliciously producing toxins. This is not true of oral bacteria associated with caries. When acid is produced in the plaque, it becomes neutralized because it dissolves out enough of the calcium salts from the tooth. Now, I am not going into the chemistry of it because I do not understand all of it. Simply, the acid dissolves out calcium salts and becomes neutralized. Then the process stops for a time. The organisms are well fed. They have had their meal; they have secreted their metabolic products. All they do is wait until the next time you go and get a "coke," or a piece of candy, or anything else that can be used to produce more acid, and that goes on again and again. As a result, caries penetrates deeper until it finally reaches the dentino-enamel junction.

At the dentino-enamel junction, apparently something different happens because we are no longer dealing with a completely or almost completely inorganic material. We now have more organic material, and so when the carious process comes to the dentin it spreads out along the dentino-enamel junction, and both proteolytic bacteria and acid-forming organisms produce the next stage of the carious process. However, the initiation of the carious process was a decalcification of the enamel by an accidental product, or an incidental product, of bacteria that simply took up their habitat in the oral cavity.

This story of the dynamic process fits in with many things that we know. As Dr. Stephan told us yesterday, every time caries is present acid is present. We have watched these things in ground and decalcified sections. Every oral pathologist has looked at enough sections so that he has seen most of these processes in series. As I said, Dietz has watched this, and he has observed changes that seem to make this a very sound theory.

There is an opposing, or different, concept of dental caries that has been presented through the years. According to this concept caries is primarily a proteolytic rather than a decalcifying process. This viewpoint is based on the idea that in the enamel we have organic material. This is true, but organic matter represents only about 2 per cent of the enamel. However, the jellyfish is only about 2 per cent organic material and the rest is just water. The organic material is chiefly in the interprismatic substance that is between the enamel rods, and it also forms so-called "lamellae," which some people like to regard largely as cracks instead of "lamellae." There are organic material on the surface, the cuticle, which may or may not be the product of epithelium, and tufts and spindles near the dentin. The proponents of the proteolytic theory say that caries progresses primarily in the organic pathways rather than the way that we have seen through the years. There is very little evidence, in my concept, to support this theory. The proponents of this theory occasionally find a section that shows caries at the dentino-enamel junction with a lamella coming into it, and no evidence of caries above it (toward the surface). That is their chief evidence, but it is very poor evidence because if sections are cut through the edges of the conelike or pyramidal areas of caries, the same picture can be reproduced as an artifact. In order to prove this concept of the carious process it would be necessary to have serial sections so that we would know that there wasn't any caries above the dentino-enamel junction. I just cannot believe that this concept fits in with what has been observed for years by oral pathologists and what has been presented as the work of a great many capable men. The proteolytic idea is not new, and the burden of the proof lies on the individuals who want to substitute it for the decalcification theory. They must show that the process they describe actually occurs in a majority of cases. There is no scientific paper, that I know of, which shows that this process actually takes place. Unfortunately, the proponents of this theory say it has been proved, unmistakably, but they have never presented that undeniable proof. But, the scientists who presented the decalcification theory built it up slowly and carefully over many years.

Now, I had to give you that concept of caries so we would all know what we are talking about. We are not speaking of a disease like tuberculosis, like diphtheria or Vincent's infection, but we are talking about a disease that is produced by organisms living in the oral cavity and outside the body, and which have a metabolic process which is destructive to part of their environment.

With that concept in mind we can discuss the relationship of systemic factors and dental caries. I think that I might say that the title of this paper should be "The Minor Role of Systemic Factors in Dental Caries Etiology."

THE EFFECT OF SYSTEMIC DISEASE ON THE CARIES PROCESS—PREGNANCY, ENDOCRINOPATHIES, OSTEOMALACIA, EMOTIONAL DISTURBANCES, AND OTHERS

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Is there any acceptable evidence of a direct relationship between dental caries and systemic disease? Although the dental and medical literature contains many seemingly erudite discussions of the relationship between such diseases as endocrinopathies, tuberculosis, diabetes mellitus, and nutritional deficiencies, as well as pregnancy, psychoses, and semantics, the evidence is scanty and the theories are of gossamer. In the first place, it must be understood that caries is practically a universal disease. Certainly, individuals with tuberculosis, diabetes, hyperpituitarism, psychoses, and who are pregnant can be expected to have dental decay, just as their more healthy fellows. Likewise, individuals with these diseases have common colds, heart disease, and cancer, but do we lay the blame for these common diseases on pregnancy or endocrine disease?

Who goes around and says that people who have common colds have them because they are pregnant? Anyone may get a cold and many women get pregnant. Nearly everyone gets dental caries and, again, many women get pregnant. The pregnant woman has dental caries; the person who has indigestion has dental caries. To attempt studies of that type is a very, very difficult problem. Correlations with a universal disease are hazardous.

It might be well to consider some relations between specific extra-dental diseases and caries. Among these are the effects of radiation, pregnancy, diabetes mellitus, tuberculosis, endocrinopathies, psychic trauma, and nutritional deficiencies.

RADIATION

Regato[21] pointed out the rapid destruction of the teeth following deep radiation therapy of the pharynx and larynx, even without direct radiation of the teeth. This striking destructive process appears as rampant caries, usually at the gingival margins of the teeth and often results in "amputation" of the teeth at the gingiva. This change may often offer a clue to some of the so-called systemic effects. Radiation therapy may alter the saliva qualitatively or quantitatively. Some of the cells of the salivary glands may be destroyed by the selective cytolethal effect, or the general activity of the glands may be reduced as a result of a more diffuse effect. Evidence to support this idea is seen in those mouths where the teeth disintegrate following radiation therapy despite protection of the teeth by lead shields. Lead shields are put over the teeth, the area is radiated, and the teeth become destroyed in a matter of a couple of years. In treatment of carcinoma of the upper air passages destruction of the tumor is not expected until an "epidermicidal" effect is seen on the normal mucous membrane.[1] Here then, is an extradental factor, radiation, which in-

directly leads to rampant caries by its effect on the saliva. Others[7, 13] have demonstrated clinically and experimentally that reduced salivary flow increases caries activity. Does this mechanism explain some of the so-called systemic effects? May the lack of "immune factors" in the saliva result from suppressed secretion? Is this a purely quantitative effect, or could it be qualitative? The complete answers to these questions remain for future investigations.

PREGNANCY AND LACTATION

Pregnancy is traditionally a cause of dental caries. Recent opinions and studies[6, 15, 20, 22, 24, 26, 30] have shown this to be a mere myth, perpetuated by the fallacious logic that because pregnant women develop dental caries, pregnancy causes caries—an example of the *post-hoc ergo propter hoc* principle. We have caries; we have pregnancy. Therefore, the caries was caused by the pregnancy. Now, we could have it the other way: Because they got the caries first the pregnancy must have been caused by the caries. It is just as logical.

Ziskin and Hotelling,[30] Mull, Bill, and Kinney[20] and Starobinsky,[26] have shown that caries does not occur at a greater rate during pregnancy. They have shown a very simple fact, that pregnant women have caries just the same as their nonpregnant counterparts. I have often wondered if these people who think pregnancy is a cause of dental caries—a principal cause of dental caries—ever have any men as patients, or perhaps that has a double-barrel answer. It works for the pregnant men as well as the pregnant women.

Schour,[24] Robinson,[22] and Burket,[6] through dental embryology and physiology, have pointed out barriers to dental calcium withdrawal in pregnancy. Deakins and Looby[11] showed that the chemical composition of teeth was not altered during pregnancy, and Klein,[15] experimentally, demonstrated the inaccuracy of the concept of pregnancy, per se, being a cause of caries.

Lactation is in the same general category as pregnancy as a cause of caries.[20]

DIABETES MELLITUS

Magitot,[17] Grunert,[12] and Lederer,[16] all in the preinsulin era, observed increased or rampant caries in diabetics. Boyd[3] has maintained that caries is reduced in controlled diabetics, and Ziskin, Siegel, and Loughlin[31] found no difference in caries experience of diabetic children in a camp and nondiabetic children living in the same area. Cohen[8] found an accelerated rate of caries with age in a group of twenty-seven young diabetics studied over a period of time. Investigators have been unable to show consistently increased salivary sugar in diabetics, but diabetics do have reduced salivary flow. Is simple xerostomia the factor that caused rampant gingival caries in uncontrolled diabetics? Do we have any uncontrolled diabetics available for study, or are we considering the results of a therapy rather than the results of a disease?

TUBERCULOSIS

Tuberculosis is another disease that has been called caries-producing, and we come back to the fact that people with tuberculosis have caries and people without tuberculosis have caries.

Tanchester and Sorrin[27] after a study of 1,652 tuberculous patients found "no pathognomonic dental or periodontal signs of tuberculosis." These authors and Miller[19] noted instances of decalcification of the teeth in tuberculous patients who used lozenges to relieve mouth dryness. Again, was there a relationship here between xerostomia and caries as well as from the acid lozenges? It is interesting that Broderick,[5] a strong advocate of systemic causes for dental caries, found 25 per cent of the teeth of tuberculous individuals to be "splendid" and 9 per cent "very good," while only 18 per cent were "excessively carious." And that in England!

ENDOCRINOPATHICS

Many of the glands of internal secretion have been indicted as the cause of caries because caries occurred in individuals with endocrine disease. Shour and Massler,[25] in their review of endocrines and dentistry, I think, summarized this as well as anyone. They considered the present status as follows:

"Caries is so widespread that the coincidence of caries with a given endocrine dysfunction can be high and yet have no causal relationship. The impression of some that a high incidence of caries occurs in cretinism and pregnancy has been repeatedly disproved upon further careful clinical examination."

And we get that unfortunate situation of a man in the field of endocrine disease who has a patient, let us say, with hyperthyroidism. This endocrinologist may see caries and write a paper claiming that patient so-and-so had hyperthyroidism and dental caries was caused by it. Now, as a matter of fact, endocrinopathy probably had nothing to do with the caries. I have seen an instance of a patient who had parathyroid disturbance with massive destruction of the bone of the jaw but with no destruction of the teeth. That doesn't prove that one can't have caries with hyperparathyroidism, but it means that causal relationship has not been shown.

NUTRITIONAL DEFICIENCIES

Collins, Jensen, and Becks[9] showed that essential food elements were no more deficient in a group of 122 students with rampant caries than they were in a group with practically no caries. Taylor and Day[28] found only 1.54 caries per mouth in twenty-two East Indian women with osteomalacia. Here were women with calcium disturbance with no increase in dental caries. In fact, they had less than we would expect here. These women were able to give birth to children, and in 144 teeth of the rachitic children of these women with osteomalacia, Taylor and Day found only two cavities. In a further study of 200 boys of deficient mothers living on deficient diets, 55.5 per cent had no caries of the permanent teeth and 49 per cent were entirely caries-free. The possibility of fluorine being active here does not detract from the findings. Mann, Dreizen, Spies, and Hunt,[18] in a careful study of 124 malnourished patients and 99 patients free from nutritional disease, found that only 30.5 per cent as much caries occurred in the malnourished group. Bacteria require nourishment as do higher an-

imal forms. The acidogenic bacteria of the mouth appear to require some of the dietary essentials of man. The effect on the bacteria seems to be more profound than that on the teeth, through systemic activity, and caries is thus arrested.

That is very simple to understand. The bacteria live on food. If man does not get food, they do not get food. These individuals down in Alabama were hungry and their bacteria were hungry. The starved bacteria didn't produce the acid to start the carious process. Bacteria require nourishment just as we do, and, therefore, caries is arrested. Man has a dietary disturbance, and it affects the bacteria more than it affects the human being, and caries ceases. This finding should not be considered as a plea for general malnutrition as a caries-control measure but serves to illustrate that good nutritional status is not as important to the erupted tooth as it is to many other organs.

PSYCHIC TRAUMA

Briggs[4] attempted to explain caries as a result of psychic trauma leading successively to endocrine imbalance, diminished salivary calcium, lowered salivary pH, and thus, the caries. To this, Barrett[2] adds semantogenic symptoms. Perhaps some instances of caries may be partially controlled by psychic reactions and decreased salivary flow but to consider this a constant or important factor is not justified. Since more than 75 per cent of all persons examined at medical centers in the United States are found to be suffering from some functional nervous disorder or emotional conflict,[22] and since almost 100 per cent of the population suffer from dental caries, correlations are misleading and statistical analyses would be inconclusive at best.

INFECTIOUS DISEASES

Increased rates of caries sometimes observed after infectious diseases may be the results of neglected oral hygiene, of reduced activity of the cheeks, tongue, and lips, of temporary xerostomia, or of prejudiced impressions. Careful statistical studies of individuals with infectious disease should be undertaken. But again, here we have a rapid disease—a rapid infectious disease and a very slow carious process, and to try to make correlations is extremely difficult.

HEREDITY AND CONSTITUTION

Hunt, Hoppert, and Erwin produced a caries-resistant strain of rats, showing that genetic factors can influence dental caries. Klein[15b] has attempted to show that there is some relationship between the carious rate of children and their parents. I believe it can be stated that in the dental caries field environmental factors overshadow constitutional factors in man. That doesn't eliminate constitutional or heredity factors from possible influence. The environmental factors simply overshadow the constitutional factors in man.

GENERAL HEALTH

Hurme[14] has attempted to correlate health history, physical measurements, and heredity with dental caries. He studied fifty-four dental students and

found no well-marked correlations between neglected and damaged teeth. However, by comparing the ends of the series he believed a relationship was shown; i.e., the ten healthiest men had an average of 16.1 carious teeth and the ten sickliest had 18.8 carious teeth. There were twenty-seven extractions in the first group and twenty-six in the second. It is doubtful if statistical analysis would place these differences outside the realm of pure chance.

Cunningham,[10] on the other hand, found no thoroughly reliable relationships between the damaged teeth of 11,117 women students and their histories of illness, menstrual functions, and physical measurements. Cunningham's material is presented so that one may recheck it. There was no relationship between the health and the caries history in these 11,000 women. Certainly, the waiting room of the usual dental clinic is not occupied by a fleet of physical derelicts but rather presents a cross-section of the population, some healthy, some ill, and many in the middle of the pond. Slight physical defects are common findings and dental caries is even more frequently found. It seems futile to attempt correlation. It isn't possible to correlate any of those general health factors with caries when almost everyone has caries. It is a practical impossibility.

SUMMARY

Systemic diseases, per se, do not cause dental caries. Disturbances that may result in xerostomia, such as diabetes mellitus, certain infectious diseases, and radiation at the cervical or maxillary region, may result in lowered resistance to dental caries through a qualitative or quantitative change in the saliva. Nutritional disturbances of certain types appear to reduce the occurrence of caries, probably through nutritional effects on the bacteria.

REFERENCES

1. Ackerman, L. V., and del Regato, J. A.: Cancer, St. Louis, 1947, The C. V. Mosby Company, pp. 121-122.
2. Barrett, L. G.: Evaluation Disorders and Caries—Semantogenic Symptoms, *J. A. D. A.* **26**: 1819, 1939.
3. Boyd, J. D.: Long Term Prevention of Decay Among Diabetic Children, *Am. J. Dis. Child.* **66**: 349, 1943.
4. Briggs, E. F.: Psychogenic Factor in Dental Caries, *D. Digest* **50**: 115, 1944.
5. Broderick, F. W.: Dental Medicine, St. Louis, 1939, The C. V. Mosby Company, pp. 476 and 480.
6. Burket, L.: Oral Medicine, Philadelphia, 1946, J. B. Lippincott Company, pp. 292-294.
7. Cheyne, V. D.: Effect of Selective Salivary Gland Extirpation Upon Experimental Dental Caries in the Rat, *Proc. Soc. Exper. Biol. & Med.* **42**: 587, 1939.
8. Cohen, M. M.: Clinical Studies of Dental Caries Susceptibility of Young Diabetics, *J. A. D. A.* **34**: 239, 1947.
9. Collins, E. O., Jensen, A. L., and Becks, H.: Studies of Caries Free Individuals. II. Is an Optimal Diet or Reduced Carbohydrate Intake Required to Arrest Dental Caries? *J. A. D. A.* **29**: 1169, 1942.
10. Cunningham, R.: Disease, Menstrual Experience and Physical Measurements of Young Women, *J. D. Res.* **14**: 439, 1934.
11. Deakins, M., and Looby, J.: The Effect of Pregnancy in the Mineral Content of the Dentin of Teeth, *Am. J. Obst. & Gynec.* **46**: 265, 1943.
12. Grunert, A.: Cited by Cohen.[14]
13. Gurley, W. B.: Unilateral Dental Caries, *J. A. D. A.* **26**: 163, 1939.
14. Hurme, V. D.: Influence of Systemic Disease, Metabolic Disturbances and Constitutional Irregularities on the Incidence of Dental Defects, *J. D. Res.* **15**: 224, 1936.

15a. Klein, H.: The Effect of Pregnancy on the Incidence of Tooth Decay, *Dental Cosmos* **77**: 864, 1929.
b. Idem, Fluoride Water and Caries in Parents and Offspring, *Public Health Rep.* **62**: 1247, 1947.
16. Lederer, W. J.: Cited by Cohen.[14]
17. Magitot, E.: Cited by Cohen.[14]
18. Mann, A. W., Dreizen, S., Spies, T. D., and Hunt, F. M.: A Comparison of Dental Caries Activity in Malnourished and Well Nourished Patients, *J. A. D. A.* **15**: 244, 1947.
19. Miller, S. C.: *New York J. D.* **4**: 30, 1934.
20. Mull, J. W., Bill, A. H., and Kinney, F. M.: Variations of Serum Calcium and Phosphorus During Pregnancy. II. Effect on Dental Caries, *Am. J. Obst. & Gynec.* **27**: 679, 1934.
21. del Regato, J. A.: Dental Lesions Observed After Roentgenotherapy in Cancer of the Buccal Cavity, Pharynx and Larynx, *Am. J. Roentgenol.* **22**: 404, 1939.
22. Robinson, H. B. G.: Dental Caries and the Metabolism of Calcium, *J. A. D. A.* **30**: 357, 1943.
23. Sadler, W. S.: Mental Mischief and Emotional Conflicts St. Louis, 1947, The C. V. Mosby Company, p. 5.
24. Schour, I.: Calcium Metabolism and the Teeth, *J. A. M. A.* **110**: 870, 1938.
25. Schour, I., and Massler, M.: Endocrines and Dentistry, *J. A. D. A.* **30**: 595, 753, 943, 1943.
26. Starobinsky, I.: Beobachtungen über Zahncaries bei Schwargeren und die Wasserstoffionenkonzentration in ihren Speichel, *Deutsche Monatschr. f. Zahnh.* **47**: 238, 1929.
27. Tanchester, D., and Sorrin, S.: Dental Lesions in Relation to Pulmonary Tuberculosis, *J. D. Res.* **16**: 69, 1937.
28. Taylor, G. F., and Day, C. D. M.: Osteomalacia and Dental Caries, *Brit. M. J.* **2**: 221, 1940.
29. Walker, A.: In Dental Caries, ed. 2, *Am. D. Assoc.*, Chicago, 1941, pp. 226-227.
30. Ziskin, D. E., and Hotelling, H.: Effects of Pregnancy, Mouth Acidity and Age on Dental Caries, *J. D. Res.* **16**: 507, 1937.
31. Ziskin, D. E., Siegel, E. H., and Loughlin, W. C.: Diabetes in Relation to Certain Oral and Systemic Problems, *J. D. Res.* **23**: 317, 1944.

DISCUSSION

DR. EASLICK.—Thanks very, very much. Now, since he is practically the last speaker in the group to explore the process of caries, I rather suspect that some of the other speakers will have to get into the process to carry out their assignments. I think it would be smart here to pause and ask for discussion.

Are there any in this audience who would like to discuss Dr. Robinson's paper, or who would like to quiz, or ask questions?

DR. DONALD A. KERR.—I think Dr. Robinson has covered the field adequately and very well, there doesn't seem to be much need for discussion.

DR. EASLICK.—You mean he came out with a negative statement?

DR. KERR.—That is right.

DR. EASLICK.—Dr. Zander?

DR. HELMUT A. ZANDER.—It is too bad that I want to discuss it after this speech by Dr. Kerr, but Dr. Robinson told us about the bacteria and the plaque, and he then told us that once these bacteria reach the dentin, they may be proteolytic bacteria. Is it unreasonable to suspect that it is quite possible that in this plaque we have both bacteria which like protein as food as well as carbohydrate? That is my first question.

DR. ROBINSON.—I do not believe there is anything about it that would contradict that idea.

DR. ZANDER.—Now, if the proponents of this theory state that caries is essentially a proteolytic process, is it not possible that if this plaque should have its origin or its location on one of these cracks or lamellae, as you like, that proteolytic bacteria, which, as we just stated, might very well be in this plaque may start getting into this crack which contains cells

or some protein material, and if they do, is it not possible then that the metabolism of these may have other end products other than acid, and that some of those end products may be some of the stains we heard about recently, and that we heard about a hundred years ago? I am talking about some of the yellow and brownish stains that have been mentioned in places as far away as the dentino-enamel junction, even though the enamel was not attacked by acid, or dissolved, or destroyed. Could it not possibly be that we can get a yellow stain here an as end product of some bacteria other than those forming acid?

DR. ROBINSON.—You mean without any evidence of any change through the enamel?

DR. ZANDER.—Yes.

DR. ROBINSON.—I doubt it. That yellow stain has recently become a very interesting subject. It was claimed by Gottlieb that he could not produce artificial caries because his artificial lesions did not have the yellow stain. He set up teeth in test tubes with certain combinations of sugar and saliva and produced decalcification, but he said that it was not caries because he had no yellow stain. I repeated the experiment, but when I did I was sitting smoking my pipe in the laboratory, and I took a pipette and blew smoke down through the solution and my caries or artificial decalcification had a yellow stain. I can't see how we could have a process in the mouth that would make the tooth capable of being penetrated without having some stain enter from the mouth. The calculus in the mouth is usually stained yellow. I think that stain is just something that there is too much fuss about, and you don't have to go and find some bacteria going down an organic pathway to produce that particular stain. I do not believe that your suggestion necessarily follows, Dr. Zander.

DR. ZANDER.—All right, I quite accept your explanation. We will just go one more little step further since we mentioned the name of the proponent of this theory, also states on the second page—on the first page he says proteolytic process and on the second page he will say it is not possible that this goes on with an acid process, and if you look at the pictures that are shown you find that in almost all instances you have some signs of acid destruction of tooth substances. And then I was wondering if he, as the proponent of this old theory, and what we think today are really so very far off if we take the second page in the book—and I don't mean to take this literally, it may be pages 101 and 102 where he stated the proteolytic process, is it not possible that since all types of bacteria are possible in this plaque that there are some bacteria that actually have other metabolic processes than acid formation, and some of those metabolic processes may have been the stain, or the stain may have gotten in from the outside, so that there is at the same time proteolysis and acid formation going on, and if you look long enough you get a fine, wide crack or lamella and it will actually show bacteria in that lamellae? And I think he has shown in his book lamellae which contain bacteria.

DR. ROBINSON.—I quite agree with you on that, there are all sorts of bacteria in the plaque and in the lesion that may produce some other end products. Actually, caries may progress down the lamellae, but that doesn't happen very often. You know how many sections we see in which decalcification predominates compared to those where lamellar caries is present. I admit that lamellar caries might occur occasionally, but I think that it is a relatively rare thing, and that isn't the way we should direct our control ideas. We should direct them toward the more universally accepted concept, the more common process. Also, the fact remains that in caries itself the reaction is predominantly acid. As Dr. Stephan showed, the carious lesion is predominantly acid. I think the evidence indicates very strongly that the acidogenic, so-called chemico-parasitic, theory is the concept that is acceptable, and this other concept describes a process that may take place on rare occasions. The action may be through some different process from that suggested by Gottlieb; that is, the actual penetration may be bacteria growing alongside the lamellae. If we had an organic pathway through the enamel when acid decalcification or bacteria come up against that organic material, the bacteria, if they were decalcifying enamel, would have to follow down along the lamella

because that would be a barrier to their spreading beyond. I have no complaints against the proponents of that theory. They say that one process happens occasionally, that the other occurs most of the time.

DR. EASLICK.—Dr. Stephan, can you say anything about the pH of the plaques that would interfere with the growth of proteolytic bacteria?

DR. ROBERT M. STEPHAN.—Well, I don't know. Of course, there are a lot of bacteria that are not proteolytic. I don't believe, for example, that lactobacilli, and so forth, secrete proteolytic enzymes, but I don't suppose there is any reason why some proteolytic bacteria could not grow down there. I think it would have to be investigated and shown otherwise. There is no reason to conclude either way.

DR. EASLICK.—There's a question in the back of the room.

DR. BASIL G. BIBBY.—I wonder whether Dr. Robinson would care to explain two reports which have rather worried me? One from Switzerland and one from "Christina" in which correlations were made between the caries activity and growth of clinical groups, and I think this might be the key point in which there was a very definite and very satisfactory correlation between the amount of dental caries and the number of births of the group. The group had anything up to eighteen children which made up the study groups.

And the other point which, I think, Dr. Robinson seemed to slight is the question of why is there an apparent correlation between certain age periods and the activity of dental caries? There are two strong men that Dr. Robinson can knock down.

DR. ROBINSON.—In the first place I will consider the eighteen pregnancies. Where did they occur?

DR. BIBBY.—Switzerland.

DR. ROBINSON.—All that in Switzerland? I do know that a number of individuals put animals on repeated pregnancies, and they were not able to increase the rate of dental caries in that way. I think that any individual who would have eighteen pregnancies would certainly have many other factors other than pregnancy that might enter the caries picture. That would have to be considered an extraordinary example to have that relationship. And what was your second point?

DR. BIBBY.—The relationship between the activity of caries and certain age groups.

DR. ROBINSON.—I am not certain there is a correlation between the activity of caries and age groups when we start analyzing the facts. Young individuals do have more caries, or appear to have more caries, than people at older ages. That is a very simple thing to explain. There are only a given number of susceptible areas in the mouth. Let us just consider the eight teeth in one quadrant of the mouth. After the first molar decays we reduce the number of caries-susceptible areas, and as the second molar decays they are reduced again. This is true when the premolars decay and, as the individuals gets older, there are just less places to have dental caries than when they were young. The susceptible teeth became carious shortly after they erupted and there are susceptible teeth until an individual is 18 or 19 years old. The so-called accelerated rate is simply decay of susceptible teeth. When the number of susceptible areas is reduced, the rate of caries slows down, not through any constitutional change, but through a reduced opportunity for decay. Now, that may be oversimplifying the problem, but that has always seemed to me as a logical explanation of the so-called accelerated rate of caries in adolescents.

You may also be thinking, Dr. Bibby, of the fact that there are times in people's lives when it seems they have no caries, and then they have caries, and may cease again. I think that problems remain in the field of Dr. Williams. There may be certain immune factors which are in the saliva. There may be local environmental factors that may pop up in this individual's saliva, it may be lost, and it may come and go. It may be a transient thing. As

Dr. Williams showed yesterday, when an individual is artificially immunized he may lose that immunization against lactobacilli very rapidly. An individual may acquire an immunity, may acquire new strains of lactobacilli. Rosebury demonstrated that the pilot biscuit brought to Eskimos started their dental caries. Now, did the pilot biscuit start the dental caries, or did somebody have to bring the organisms with the pilot biscuit? That is something that fits in with this general problem. Can you just say that the carbohydrate, per se, was the cause? If those individuals had received the pilot biscuit with no white men allowed to contact them, would they have acquired caries? You see, maybe organisms that are transient may be a factor. Does that help explain?

DR. BIBBY.—That complicates the question very nicely.

DR. WALLACE D. ARMSTRONG.—I would like to discuss a point by asking a question, which I am sure that the clinical investigators may be able to answer in proximate fashion. I would like to know what is the time relations in the development of the carious process? Now, I realize that that will be extremely variable, but how long a time is required for this process that you have outlined to progress in its initial stages to tooth destruction, or rather, toothache?

DR. ROBINSON.—I don't know. I wish I did, Dr. Armstrong. If you are going to ask me to make a guess, and this is a pure guess, I would say that could happen in anywhere from a few weeks to several years, because there are many different types of caries. There is so-called "acute" caries, which is rather rapid, and so-called "chronic" caries, which is very slow. A study that someone might undertake is to classify caries and find if we are talking about the same thing all the time. You will recall, if you go back in literature, that at one time there were just diseases of the chest, and then medicine got to a place where pneumonia and tuberculosis were recognized. Methods improved a little bit and pneumonias were classified, and then the bacteria associated with pneumonia, and finally a lot of different diseases came out of it. It is just possible—I don't like to throw this out to you because it might sound confusing—but it is just possible that what we call dental caries may represent several different diseases, and they may all have different rates. If somebody does know the relation of the time factors, I would like to know it.

DR. PHILIP E. BLACKERBY.—Dr. Robinson, accepting your explanation that caries of the enamel is primarily an acid decalcification, would you comment on the statement that is frequently made, that caries as it occurs in the mouth, and as it is produced artificially in the laboratory by the use of acid, presents two radically different microscopic pictures?

DR. ROBINSON.—The statement is made quite often that nobody has ever produced the carious process artificially. Now, I think what they should say is, that nobody has produced the complete process; that is, going on to the proteolytic action that appears to accompany caries in the dentin. As far as the process in the enamel is concerned I think a number of people have seen that. I think W. D. Miller, for one, saw that. At Michigan, here, they have demonstrated that type of thing. I think Dr. Bunting demonstrated it, and I am certain that Dietz was looking at it under the microscope. The carious process, as we see it, has been produced up to the point where there should be a sort of a double action. In other words, the decalcification part of it has been reproduced artificially, but I don't think the entire process has been produced artificially.

DR. BLACKERBY.—Will you explain the difference then on the basis of the artificial production that the entire inorganic process involves the dissolved portion of the enamel and that the difference in the picture is entirely a question of difference by omission of the proteolytic portion?

DR. ROBINSON.—I think that is a pretty fair statement of it, yes.

DR. EASLICK.—I wonder if Dr. Philip Jay would like to comment on the acid organism, whether it continues in old people like Bibby and Robinson here, or whether they do drop or not?

DR. PHILIP JAY.—Of course, the lactobacillus count may increase in individuals at any age. Why, we are not sure, but very often it can be attributed to a change in dietary habits in which sugar consumption is increased.

Reverting to the last question about the dissimilarity between experimentally produced lesions in test tubes and those that occur in the mouth. I think one other factor ought to be considered: You are getting a quantitative difference there, that is considering just the acid action. In the test tube you are getting a mass action, and I think that is perhaps why it is shelled out the way it is. As Dr. Stephan showed yesterday, the acid production takes place, in spurts. It isn't a constant action.

DR. LEONARD S. FOSDICK.—I would like to make an additional comment on why a natural carious lesion is different from an artificial one, if I may come up to the board here?

In a natural carious lesion we have an additional factor where we have the pulp in which there is an osmotic pressure of around 6.8 atmospheres, and on the plaque we may have an osmotic pressure as high as around 45 to 50 atmosphere, simply by the ingestion of rather concentrated sugar solutions. Now, that would explain why we have the type of inverted cone in the living tooth. In other words, the acids would tend to progress in toward the pulp. If the caries were up here, it would come down this way. It would follow the lines of least resistance until it hit the rather spongy dentin. That is, the dentin could be considered more spongy in relation to the enamel, or less crystalline and it would act exactly the same way, for instance, as pouring water on a sponge through a vacuum. For instance, if we take an ordinary vacuum and a funnel, and stick a vacuum on here and place a sponge in it, the water would hit the sponge and go sideways as well as toward the bottom, and I think the same type of affair acts in the enamel and dentin. You would have more straight lines toward the dentin, and when it hit the dentin it would diffuse sideways as well as toward the pulp. Now, artificial caries in an extracted tooth should present the appearance of caries in a pulpless tooth, and I believe there is some evidence to indicate that pulpless teeth decay more like an artificial carious lesion than the regular living teeth.

DR. OLIN E. HOFFMAN.—Can you tell us at what pH level proteolysis may occur?

DR. EASLICK.—Dr. Stephan, do you want to take that?

DR. ROBERT M. STEPHAN.—Well, there are different proteolytic enzymes that have their activities at different pH levels. You have pepsin, for instance, that is active at a very low pH, and trypsins that are in alkaline range and other enzymes around pH 4 and pH 5. In other words, different enzymes have different pH levels, and presumably some proteolytic enzymes could cover the entire pH range and occur in living organisms.